



National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

UNIVERSITY OF ALBERTA

**AN NMR EVALUATION OF WATER DIFFUSION AND
MAGNETIZATION TRANSFER IN NERVE**

BY

CHRISTIAN BEAULIEU

A thesis submitted to the Faculty of Graduate Studies and Research in
partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
MEDICAL SCIENCES - BIOMEDICAL ENGINEERING.

Edmonton, Alberta

FALL 1995



National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

THE AUTHOR HAS GRANTED AN
IRREVOCABLE NON-EXCLUSIVE
LICENCE ALLOWING THE NATIONAL
LIBRARY OF CANADA TO
REPRODUCE, LOAN, DISTRIBUTE OR
SELL COPIES OF HIS/HER THESIS BY
ANY MEANS AND IN ANY FORM OR
FORMAT, MAKING THIS THESIS
AVAILABLE TO INTERESTED
PERSONS.

L'AUTEUR A ACCORDE UNE LICENCE
IRREVOCABLE ET NON EXCLUSIVE
PERMETTANT A LA BIBLIOTHEQUE
NATIONALE DU CANADA DE
REPRODUIRE, PRETER, DISTRIBUER
OU VENDRE DES COPIES DE SA
THESE DE QUELQUE MANIERE ET
SOUS QUELQUE FORME QUE CE SOIT
POUR METTRE DES EXEMPLAIRES DE
CETTE THESE A LA DISPOSITION DES
PERSONNE INTERESSEES.

THE AUTHOR RETAINS OWNERSHIP
OF THE COPYRIGHT IN HIS/HER
THESIS. NEITHER THE THESIS NOR
SUBSTANTIAL EXTRACTS FROM IT
MAY BE PRINTED OR OTHERWISE
REPRODUCED WITHOUT HIS/HER
PERMISSION.

L'AUTEUR CONSERVE LA PROPRIETE
DU DROIT D'AUTEUR QUI PROTEGE
SA THESE. NI LA THESE NI DES
EXTRAITS SUBSTANTIELS DE CELLE-
CI NE DOIVENT ETRE IMPRIMES OU
AUTREMENT REPRODUITS SANS SON
AUTORISATION.

ISBN 0-612-06184-1

Canada

UNIVERSITY OF ALBERTA

LIBRARY RELEASE FORM

NAME OF AUTHOR : Christian Beaulieu

TITLE OF THESIS : An NMR Evaluation of Water Diffusion and
Magnetization Transfer in Nerve

DEGREE : Ph.D. in Medical Sciences - Biomedical Engineering

YEAR THIS DEGREE GRANTED : 1995

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

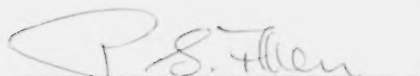
Christian Beaulieu
Christian Beaulieu
8210 - 111 St. #505
Edmonton, AB
T6G 2C7

DATE: September 15, 1995

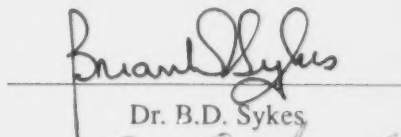
UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

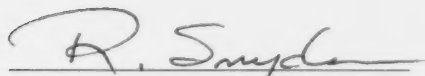
The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled AN NMR EVALUATION OF WATER DIFFUSION AND MAGNETIZATION TRANSFER IN NERVE submitted by CHRISTIAN BEAULIEU in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in MEDICAL SCIENCES.



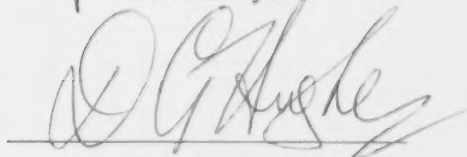
Dr. P.S. Allen (supervisor)



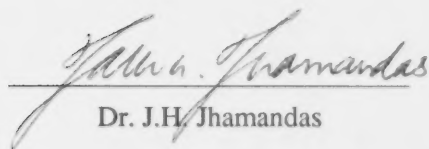
Dr. B.D. Sykes



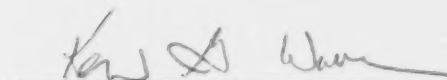
Dr. R.E. Snyder



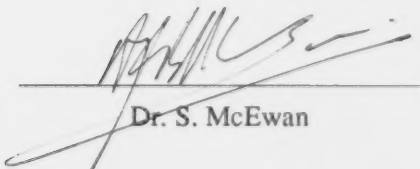
Dr. D.G. Hughes



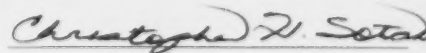
Dr. J.H. Jhamandas



Dr. K.G. Warren



Dr. S. McEwan



Dr. C.H. Sotak

Date: August 8, 1995

9

This thesis is dedicated to my family :
my mom, Ghislaine;
my dad, Gilles;
and my brother, Jason.

ABSTRACT

By providing excellent contrast of soft tissue, magnetic resonance imaging (MRI) has proven to be a useful diagnostic tool in medical imaging. MRI can provide excellent anatomical detail, yet it can also provide functional information about the tissue (e.g. blood flow, water diffusion, etc.). In order for the full potential of MRI to be realized, the influence of the physiological microstructure of tissue on the various tissue water parameters such as transverse relaxation, longitudinal relaxation, diffusion, and magnetization transfer must be understood. Our investigations focused on the diffusion of water and, to a lesser extent, on the magnetization transfer between water protons (i.e. hydrogen nucleus) and macromolecular protons in excised nerves that were used as models of white matter.

Anisotropic water diffusion is observed in normal nerve and white matter. Myelin was hypothesized as the source of this anisotropy, but its role remained experimentally unproven. Therefore, the relative importance of the major structural components of nerve to anisotropic diffusion was evaluated by measuring water diffusion in various simplified models of white matter. The role of myelin was cast into doubt by the observation that nonmyelinated and myelinated nerves of the garfish had similar degrees of anisotropy. Further studies eliminated microtubules, fast axonal transport, neurofilaments, and local magnetic-susceptibility-induced gradients as primary sources of anisotropy. Hence, only membranes, either in the form of myelin (if present) or axonal membranes, remained as the dominant source of anisotropic diffusion of axonal and inter-axonal water in nerve and presumably in white matter. We subsequently showed that anisotropy was significantly reduced in

peripheral nerve that had undergone Wallerian degeneration, thus potentially providing a method of monitoring gross nerve degeneration.

White matter shows greater magnetization transfer than gray matter, *presumably* due to the abundance of myelin in white matter. To further investigate the contributions of myelin, several magnetization transfer properties of water in myelinated and nonmyelinated nerves of the garfish were compared. The degree of magnetization transfer at steady-state was similar for all of the nerves, implying that MT is quite nonspecific.

ACKNOWLEDGMENTS

I would like to start off by thanking my supervisor Dr. Allen, I mean, Peter, for providing superb leadership and guidance throughout the course of this work. However, he also gave me the freedom to pursue my own ideas, and for that I am grateful. My only regret is that I wish that I could have "picked his brain" to a greater extent. A warm thanks is extended to my supervisory committee for their useful comments and suggestions. In particular, I would like to thank Dr. Brian Sykes for writing numerous letters of support which prolonged my MRC funding throughout the duration of my degree. The collaborations with Dr. Richard Snyder have been extremely fruitful. It was a treat to discuss, even briefly, science with Dr. Christopher Sotak, who acted as my external examiner. Dr. Zoltan Koles (a.k.a. hockey genius) graciously served as chairman and kept back the "hounds" for both my candidacy exam and final defense. Useful discussions with Dr. Gang Zhu and Dr. Chris Hanstock are appreciated. Finally, with respect to the academic circles, I would like to thank Dr. Jim Peeling (U. of Manitoba) for suggesting Peter's lab as a potential site for graduate work, and Dr. Ted Schaefer (U. of Manitoba) for introducing me to NMR during my undergraduate years. The three summers that I worked for Dr. Schaefer changed the course of my life.

To nobody's surprise, this thesis relied on the expertise and help of several individuals. I have been privileged to work with so many fantastic people. I thank Joe DeGiorgis (Calamari Inc.) at the Marine Biological Laboratory in Woods Hole, MA for teaching me how to dissect giant axons from squid and to Phil Turk at the Marine Biomedical Institute in Galveston, TX for supplying live squid. The quality of the electron micrographs are due to the expertise and tutelage of Dr. Ming Chen (Surgical and Medical Research Institute). Maxine (Neurosurgery) lent an excellent microscope for the experiments on squid. Mark of Lab Animal Services did a great job keeping the garfish alive over a long period of time and he had the courage to scoop the garfish out of their tank. Isao Yamamoto constructed most of the plexiglass/teflon formers. Ivy Ho, Ion Bucliu, and Mark Astridge were instrumental in the computing aspects of this work. I cannot thank Rosie Vishram enough for her help with various nerve dissections, the action potential measurements, and general information about nerves. It is always a pleasure to work with someone so kind and generous. Narc Ouellette constructed some of the hardware used for the action

potential measurements. Karim Damji designed the gradients that were essential for this work and he was always there with a thorough answer whenever I had a question that was bothering me. In addition, Karim was the first person that I went skiing with in the Rockies and he was one of the original NMR golf maniacs. Carol Tymchuk (the real powerhouse in the lab), in her calm, cool, and collected manner, has kept my papers, scholarships, and general day-to-day activities in total control for the past five years. I owe her a great deal and I thank her dearly for being so thoughtful and charitable. She is an integral part of this lab and life in Pete's lab would not have been the same without her.

Dan "*Butthead*" Doran (now we're even) is a great friend, an awesome technician, and 3-time winner of the Butthead-of-the-Year award. He taught me about the Bruker and kept it in tip-top shape (except for that crazy CDC hard drive which had a mind of its own). Furthermore, he spent endless hours (generally over a couple of large Macadamia nut coffees) helping me with every aspect of this project. One of the more memorable moments was the time spent winding, rewinding, and constructing the gradient coils. In addition to time spent in the lab, the early morning "suited-up" one-on-one lacrosse sessions, Friday after-work basketball, Sunday night hockey, the never-ending golf "competitions", a string of hockey pools, etc. with Dan have made life a lot more interesting. I blame Dan for getting me hooked on golf, particularly by letting (bull!) me beat him on the last game of the year for the last 3 years!!

Of course, where would anyone be without their fellow graduate students. The "first" generation of grad students included Alan "Multiple Quantum" Wilman, Frank "Ironman" Ye, Frances "T₂" Fenrich (more to follow), Edmund "LP" Ho, and Pat "The Cat" Stroman. In particular, Pat was a great guy to hang out with and his more noticeable features were his wild NMR ideas, his love of beer, his flight simulator abilities, and his lack of a temper, especially on a golf course (yah right). His practical jokes are unforgettable. The "second" generation of grad students included Mark "Frogman" Does and Rich "Density Matrix" Thompson (the Matlab gurus). Rivalries at golf and poker were always a blast. In general, I was kind-hearted enough to play below my abilities in order to let Mark and Rich win. Traditional Ale on Friday afternoons became a ritual. KHL hockey discussions, or should I say debates, with Mark occupied far too much time and prolonged the writing of this thesis. When we weren't doing any of the above activities, many

collaborations with the Frogman provided some interesting results. I consider myself fortunate to be friends with these two chaps.

I would like to thank all of my good friends that have not been mentioned yet for giving me a million excuses to avoid work: James Morris, future psycho MD (or is that MD for psychos) who would whack me around in karate class; Stéphane Gagner, biochemistry NMR Frenchman who has a habit of getting his butt kicked (well sometimes) at golf in the summer, at pool in the winter, or at poker anytime; Marshall Norton, private eye who enjoys ski trips to Utah and Rockies Pizzeria for that fresh mountain air; Kevin Dawson, recently married computer guru that I've known for a very long time; Paul Mayer, mass spectrometric wizard who survived undergraduate chemistry with me. I would also like to thank my sensei, John Priegert, for everything that he has taught me about karate and self-awareness.

My parents, Gilles and Ghislaine, have been always very supportive of my schooling since day one. Their generosity is greatly appreciated. I owe everything to them. They have kept me sane during the course of this thesis with numerous fun-packed and stress-relieving trips when they lived in Baden-Baden, Germany. Despite living far apart, my brother Jason and I have always made time to go skiing, fishing, golfing, or whatever.

The Alberta Heritage Foundation for Medical Research (AHFMR), the Medical Research Council of Canada (MRC), the Alberta Heritage Studentship Fund (AHSF), and the University of Alberta have given me financial support for golf, travel, and beer, I mean, food, housing, and tuition.

Finally, I must thank Frances for being so wonderful, and for tolerating my antics and peculiarities for the past 4 years (holy smokes). Her opinions and insight were invaluable during the course of this thesis. However, it is unfortunate that she has dropped to second place in the "garfish dissection skills" category.

This thesis was brought to you by the number "1" and the letter "H".

TABLE OF CONTENTS

Chapter	Page
1 Preliminary Remarks	1
2 Theory and Background.....	4
2.1 Some Aspects of Nuclear Magnetic Resonance.....	4
2.1.1 Spin and Magnetic Moments.....	4
2.1.2 Interaction of Protons with a Static Homogeneous Magnetic Field.....	6
2.1.2.1 Single Spin.....	6
2.1.2.2 Ensemble of Spins.....	7
2.1.3 Interaction of Protons with Both a Static and Time- Varying Magnetic Field	8
2.1.4 Longitudinal and Transverse Relaxation	13
2.1.5 Measurements of Diffusion by NMR.....	17
2.1.5.1 Basics of Diffusion.....	17
2.1.5.2 Diffusion in an Inhomogeneous Static Magnetic Field.....	21
2.1.5.3 Pulsed-Gradient Spin-Echo Method to Measure Diffusion	28
2.1.5.4 q-Space Imaging.....	37
2.1.6 Magnetization Transfer.....	41
2.2 Some Applications of NMR to the Study of Water in the Nervous System	51
2.2.1 Nuclear Relaxation of Water Protons in the Nervous System	51
2.2.2 Water Diffusion in the Nervous System.....	54
2.2.3 Magnetization Transfer in the Nervous System	63
3 Experimental Materials and Methods	69
3.1 Animal Models.....	69
3.1.1 Garfish	69
3.1.1.1 Description and Source	69

3.1.1.2	Excision of Nerves	71
3.1.1.3	Preparation of Nerve for NMR Measurements.....	72
3.1.1.4	Validation of Nerve Integrity and Viability	73
3.1.1.5	Vinblastine Treatment of Garfish Nerves.....	79
3.1.1.6	In-Vitro Degenerated Nerves.....	80
3.1.2	Squid	80
3.1.2.1	Description and Source	80
3.1.2.2	Excision of Nerves	82
3.1.2.3	Validation of Nerve Integrity.....	84
3.1.3	Frog.....	84
3.1.3.1	Description of Model of Wallerian Degeneration.....	84
3.1.3.2	Nerve Preparations	85
3.1.3.3	Validation of Integrity/Degeneration in Uninjured/Injured Nerves.....	86
3.2	Methodology for Measurements of Diffusion.....	86
3.2.1	Hardware.....	86
3.2.1.1	Gradient Coils.....	87
3.2.1.2	Radiofrequency Coils	90
3.2.2	Pulse Sequences and Parameters Used to Measure Diffusion	90
3.2.2.1	Basic Pulsed-Gradient Spin-Echo Pulse Sequence.....	91
3.2.2.2	Bipolar Gradient Pulse Sequence.....	96
3.2.2.3	CPMG-PGSE Pulse Sequence.....	99
3.3	Methodology for Measurements of Relaxation and Magnetization Transfer	100
3.3.1	Measurements of Transverse Relaxation Times	101
3.3.2	Measurements of Longitudinal Relaxation Times	101
3.3.3	Measurements of Magnetization Transfer	102
4	Determinants of Anisotropic Water Diffusion in Nerve	107
4.1	The Contribution of Myelin, Microtubules, and Fast Axonal Transport to Anisotropic Diffusion.....	107
4.1.1	Introduction.....	107

4.1.2	Results.....	109
4.1.3	Discussion	112
4.1.4	Conclusion.....	119
4.2	The Contribution of Neurofilaments to Anisotropic Diffusion.....	120
4.2.1	Introduction.....	120
4.2.2	Materials and Methods.....	123
4.2.3	Results.....	126
4.2.4	Discussion	129
4.3	The Contribution of Local Magnetic-Susceptibility-Induced Gradients to Anisotropic Diffusion	132
4.3.1	Introduction.....	132
4.3.2	Results and Discussion.....	134
4.3.3	Conclusion.....	140
5	Diffusion Characteristics of T ₂ Discriminated Water Pools in Nerve.....	141
5.1	Introduction	141
5.2	Results	142
5.3	Discussion.....	147
5.4	Conclusion	150
6	Changes in Water Diffusion due to Wallerian Degeneration in Peripheral Nerve.....	151
6.1	Introduction	151
6.2	Results and Discussion	152
7	Magnetization Transfer in Normal Myelinated and Nonmyelinated Nerves of the Garfish.....	159
7.1	Introduction	159
7.2	Results and Discussion	160
8	Concluding Remarks	172
	References.....	175
	Appendix 1 Schematic Diagrams of the Gradient Coils.....	195

LIST OF TABLES

Table 2.1	A summary of the long, intermediate, and short transverse relaxation time (T_2) components observed for water protons in white matter, spinal cord, and nerve.	53
Table 2.2	Diffusion coefficients measured parallel (//) and perpendicular ($\perp$) to the length of the axons (for white matter) and the anisotropic diffusion ratio of water in normal gray matter and white matter in-vivo.....	56
Table 2.3	Magnetization transfer ratios (MTR) for normal white (WM) and gray matter (GM).....	65
Table 3.1	Number and type of garfish that were studied for various experiments that are described in this thesis.	71
Table 3.2	NMR measurements of diffusion coefficients of pure water, paraffin oil, and water in celery {parallel (//) and perpendicular ($\perp$) to the length of the sample}.	95
Table 4.1	Some physical characteristics of the garfish nerves.....	109
Table 4.2	NMR measurements of diffusion coefficients of pure water, paraffin oil, and water in freshly excised, vinblastine treated, and in vitro degenerated garfish nerves.....	112
Table 4.3	One-dimensional root-mean-square displacements, λ_1 , for the diffusion of water parallel (//) and perpendicular ($\perp$) to the axonal length in freshly excised garfish nerves over a diffusion time of 28 ms.....	116
Table 4.4	NMR measurements of water diffusion parallel (//) and perpendicular ($\perp$) to the long axis in the giant axon of the squid.....	127
Table 4.5	The root-mean-square displacements in one- and two-dimensions (λ_1 and λ_2 , respectively), and the calculated diffusion coefficient anisotropy for the computer simulations of random diffusion in the absence and presence of a regular, hexagonal array of impenetrable circular barriers.....	128
Table 4.6	Measurements of the diffusion coefficients (D_{NMR}) and their anisotropy relative to the sample axis, for water protons in distilled water and in apple flesh, at two	

	different orientations of the sample axis relative to the static magnetic field ($B_0 = 2.35 \text{ T}$).....	135
Table 4.7	A summary of the mean water proton diffusion coefficients parallel and perpendicular to the nerve axis.	138
Table 4.8	An illustration of the change in water proton diffusion coefficients and their anisotropy due to a shortening of the diffusion time, t_d , in distilled water, apple, and the sciatic nerve of the frog.....	139
Table 5.1	NMR measurements of water diffusion in distilled water, and the olfactory, trigeminal, and optic nerves of the garfish at spin-echo times ranging from 70 to 450 ms.....	146
Table 5.2	Diffusion coefficients (D_{NMR}), transverse relaxation times (T_2), and longitudinal relaxation times (T_1) of distilled water, and water in 1.5% and 3% agarose gels.	149
Table 6.1	NMR measurements of diffusion coefficients of water in normal, crush injured, and tie injured sciatic nerves from the frog.	153
Table 7.1	Relaxation characteristics of water protons in 2 and 4% agar gels and in the olfactory, trigeminal, and optic nerves of the garfish.....	161
Table 7.2	Some magnetization transfer properties of water in agar gels and garfish nerves measured using an irradiation rf strength of $16.5 \mu\text{T}$ at an offset frequency of 10 kHz.	164

LIST OF FIGURES

Figure 2.1	The rotating frame representation of the motion of the net magnetization M in a field B_{eff}	11
Figure 2.2	The displacement profiles expected after an initial plane of particles undergoes diffusion for durations of $t(1) < t(2) < t(3)$	18
Figure 2.3	The log of the self-diffusion coefficient of pure water as a function of the inverse of the temperature.....	21
Figure 2.4	The rotating frame response to a spin-echo pulse sequence of two isochromats in an inhomogeneous static field.....	23
Figure 2.5	Spin-echo pulse sequence with a constant gradient, G_0	26
Figure 2.6	The basic pulsed-gradient spin-echo (PGSE) pulse sequence for measuring diffusion.....	29
Figure 2.7	A comparison of two methods of processing PGSE data for two samples with diffusion coefficients that differ by a factor of four (i.e. $D_2 = 4 \cdot D_1$).	40
Figure 2.8	The energy levels in a spin system consisting of two uncoupled spin-1/2 nuclei, I and S, in a field B_0	42
Figure 2.9	A pictorial representation of the interaction of water molecules with the macromolecular matrix (i.e. proteins, lipids, etc.) in tissue.	45
Figure 2.10	(a) The proton resonances of the macromolecular protons and the bulk water protons are illustrated. (b) The CW pulse sequence for probing magnetization transfer.	46
Figure 2.11	A simplistic schematic of the longitudinal view of a myelinated axon.....	57
Figure 3.1	Photograph of long-nosed garfish.....	70
Figure 3.2	Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised olfactory nerve of the garfish.....	75
Figure 3.3	Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised trigeminal nerve of the garfish.....	76

Figure 3.4	Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised optic nerve of the garfish.....	77
Figure 3.5	Action potentials in the nonmyelinated olfactory (a, b) and the myelinated trigeminal (c, d) nerves of the garfish at 2 and 7.5 hours post-excision.....	79
Figure 3.6	Photograph of squid.....	81
Figure 3.7	Wire arrangements and current directions that were used to generate a magnetic field gradient parallel (a) and perpendicular (b) to the static magnetic field, B_0	88
Figure 3.8	Stejskal-Tanner spin-echo pulse sequence modified with 1331 and 2662 binomial rf pulses.	91
Figure 3.9	Proton spectrum acquired by a hard $\pi/2$ rf pulse (a) and by a 1331 $\pi/2$ rf pulse (b) of a trigeminal garfish nerve that is immersed in paraffin oil.	92
Figure 3.10	Typical echo maxima obtained from a PGSE diffusion experiment.	93
Figure 3.11	Diffusion curves of pure water and paraffin oil.	94
Figure 3.12	Diffusion curves of water in vascular bundles of celery measured parallel and perpendicular to their axial length.....	96
Figure 3.13	Spin-echo diffusion pulse sequence, modified with 1331 $\pi/2$ and 2662 π binomial rf pulses, with three different patterns of diffusion sensitizing gradients.	97
Figure 3.14	CPMG-PGSE diffusion pulse sequence.....	100
Figure 3.15	The peak amplitudes of water in 0.5 mM $MnCl_2$ (aq) (a) and 10 mM $CuSO_4$ (aq) (b) as a function of the offset frequency at irradiation rf strengths of 4.7, 16.5, and 72.5 μT are compared to the direct saturation that is expected based on their T_1/T_2	104
Figure 4.1	Representative diffusion decay curves for pure water, paraffin oil, and for water in the freshly excised nonmyelinated olfactory nerve (A), the myelinated trigeminal nerve (B), and the myelinated optic nerve (C) of the garfish.	111
Figure 4.2	Transverse electron microscopy section of part of an axon in a garfish trigeminal nerve that has been immersed for 48 h in refrigerated buffer containing 0.5 mM vinblastine sulfate.	114

Figure 4.3	Electron microscopy closeup of "vinblastine paracrystals"/"microtubule crystals" (V) in an axon.	115
Figure 4.4	q-space representations of probabilities of water displacements parallel (A) and perpendicular (B) to the long axis in the olfactory (solid), trigeminal (dashed), and optic (dotted) nerves of the garfish.	119
Figure 4.5	(a) Light microscopy of a transverse section of the hindmost stellar nerve from a squid. (b) Electron microscopy of a portion of the interior of a giant axon from a squid.	122
Figure 4.6	A regular, hexagonal array of circular barriers with diameter, d , and lattice spacing, R	124
Figure 4.7	An illustration of the lattice spacings of 60 (A), 30 (B), 25 (C), and 20 nm (D) for the 10 nm diameter circular barriers which are arranged in a hexagonal array.	125
Figure 4.8	Typical results for random-walk simulations of two-dimensional diffusion in the absence {(A)-(C)} and presence {(D)-(F)} of a regular, hexagonal array of circular barriers ($d = 10$ nm, $R = 20$ nm).	129
Figure 4.9	Measurements of water proton diffusion coefficients parallel ($\parallel$) and perpendicular ($\perp$) to the length of nerve fibers as obtained with the PGSE, BGP, and BGPR sequences.	137
Figure 5.1	Mean diffusion coefficients, D_{NMR} , of water measured parallel and perpendicular to the long axis of a distilled water sample (A), and freshly excised olfactory (B), trigeminal (C), and optic (D) nerves from six garfish as the spin-echo time was varied (70, 150, 250, 350, 450 ms).	145
Figure 5.2	An example of the decay of water proton transverse magnetization in excised garfish nerves as the spin-echo time (TE) in the CPMG-diffusion pulse sequence was augmented.	146
Figure 6.1	Typical light (a) and electron (b) micrographs of a transverse section of a portion of a normal sciatic nerve from the frog.	154
Figure 6.2	Typical light (a) and electron (b) micrographs of a transverse section of a portion of an injured sciatic nerve from the frog at 28 days post-tie.	155

Figure 6.3	Typical light (a) and electron (b) micrographs of a transverse section of a portion of an injured sciatic nerve from the frog at 28 days post-crush.	156
Figure 7.1	Peak amplitudes of water in 2% (a) and 4% (b) agar gels for three rf strengths of the irradiation rf pulse at various offset frequencies.	163
Figure 7.2	Peak amplitudes of water in the nonmyelinated olfactory (a), myelinated trigeminal (b), and myelinated optic (c) nerves averaged over four garfish for three rf strengths at various offset frequencies.	167
Figure 7.3	The average peak amplitudes of water in 2 and 4% agar gels (a) and in garfish nerves (b) as a function of the duration of the irradiation rf pulse.	169
Figure A1.1	Photographs of the gradient former components.	197
Figure A1.2	The schematic diagrams of the gradient coils that were used for our measurements of diffusion.	198
Figure A1.3	Pathways for the wires that were used for the x gradient.	220

LIST OF SYMBOLS AND ABBREVIATIONS

$\perp$	perpendicular
//	parallel
90_i	radiofrequency pulse applied along the i th direction in rotating frame that produces a tip angle (Θ) of 90°
180_i	radiofrequency pulse applied along the i th direction in rotating frame that produces a tip angle of (Θ) 180°
$\langle I_z \rangle, \langle S_z \rangle$	ensemble-averaged longitudinal magnetization of spins I and S
A	echo amplitude
ADC	apparent diffusion coefficient
α_i	zeroes of a first-order Bessel function
A_0	echo amplitude in the absence of gradients
AQ	acquisition interval
ASW	artificial sea water
AWG	American wire gauge
b	gradient factor
B_1	amplitude of radiofrequency magnetic field
B_2	amplitude of irradiation radiofrequency magnetic field
B_{eff}	effective magnetic field in the rotating frame
BGP	bipolar gradient pulse sequence
BGPR	bipolar gradient pulse sequence reversed
$(B_z)_{\text{loc}}$	z component of local magnetic field
B_0	static magnetic field (customarily along z axis)
c	concentration of diffusing substance
CNS	central nervous system
CPMG	Carr-Purcell-Meiboom-Gill pulse sequence
CSF	cerebrospinal fluid
CW	continuous wave
D	diffusion coefficient
$\mathbf{D}$	diffusion tensor
Δ	time between onsets of adjacent diffusion-sensitizing gradient pulses
d	diameter of circular barriers in the simulations

δ	length of diffusion gradient pulse
D_{av}	average diffusion coefficient
$D_{barriers}$	diffusion coefficient observed in the presence of barriers
ΔE	energy difference
DNA	deoxyribonucleic acid
D_{NMR}	diffusion coefficient measured by NMR
$D_{NMR}(\perp)$	diffusion coefficient measured perpendicular to the long axis of the sample
$D_{NMR}(\parallel)$	diffusion coefficient measured parallel to the long axis of the sample
$D_{NMR}(BGP)$	diffusion coefficient measured with BGP pulse sequence
$D_{NMR}(BGPR)$	diffusion coefficient measured with BGPR pulse sequence
$D_{NMR}(PGSE)$	diffusion coefficient measured with PGSE pulse sequence
$D_{no\ barriers}$	diffusion coefficient observed in the absence of barriers
D_0	diffusion coefficient at infinite temperature
$\Delta\omega$	difference in frequency between the proton resonance and the frequency of the irradiation rf pulse
DWI	diffusion-weighted imaging
E	energy
E_a	activation energy for translational diffusion
EAE	experimental allergic encephalomyelitis
$E_{\Delta}(G)$	echo amplitude (Callaghan's notation)
EM	electron microscopy
EPI	echo-planar imaging
Φ_i	phase of i th isochromat
FID	free induction decay
FT	Fourier transform
FWHM	full-width of spectral peak at half-maximum
G	gradient strength
γ	magnetogyric ratio
G_i	gradient strength along i th direction
GM	gray matter
G_0	background or local gradient strength
G_x	gradient strength along x direction
G_z	gradient strength along z direction

$\mathcal{H}$	Hamiltonian
h	Planck's constant
$\hbar$	Planck's constant / 2π
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
H_f	free water pool of protons
$\eta_I(S)$	nOe enhancement of spin I when spin S is saturated
H_r	restricted semi-solid macromolecular pool of protons
I	spin quantum number
i	denotes imaginary component, $(-1)^{1/2}$
i	unit vector along x axis
I_0, S_0	equilibrium longitudinal magnetization of spins I and S
IOD	digital Bruker control of the current that is sent to the gradient coils
IR	inversion recovery pulse sequence
I_z	z component of spin angular momentum
j	unit vector along y axis
k	Boltzmann constant
k	unit vector along z axis
k_{app}	apparent rate constant for transfer of magnetization from H_f to H_r
k_f	rate constant for transfer of magnetization from H_f to H_r
k_r	rate constant for transfer of magnetization from H_r to H_f
l	orbital angular momentum
$\lambda_{\perp}$	root-mean-square displacement along perpendicular direction
$\lambda_{//}$	root-mean-square displacement along parallel direction
λ_1	root-mean-square displacement in one-dimension
λ_2	root-mean-square displacement in two-dimensions
λ_3	root-mean-square displacement in three-dimensions
lw	linewidth
LM	light microscopy
M	net magnetic moment
$M_{\perp}$	transverse magnetization
m_I	magnetic spin quantum number
M_0	equilibrium magnetization
M_{of}	equilibrium magnetization of H_f without pre-irradiation of H_r
MRI	magnetic resonance imaging
MS	multiple sclerosis

M_{sf}	magnitude of H_f magnetization after saturation of H_r
MT	magnetization transfer
MTC	magnetization transfer contrast
MTR	magnetization transfer ratio
M_x	x component of net magnetization
M_{xy}	transverse magnetization
M_y	y component of net magnetization
M_z	z component of net magnetization
N	number of samples
n	number of 180° rf pulses in a CPMG pulse sequence
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
PGSE	pulsed-gradient spin-echo pulse sequence
$P_s(r r', \Delta)$	conditional probability that a spin initially at r will move to r' over a time Δ
$P_{ \alpha\rangle}$	population of $ \alpha\rangle$ spin state
$P_{ \beta\rangle}$	population of $ \beta\rangle$ spin state
Q	quality factor of rf coils
Θ	tip angle of net magnetization vector
q	dynamic reciprocal space vector (used in q-space analysis)
R	lattice spacing of circular barriers arranged in a hexagonal array
r	displacement from an arbitrary origin
r	regression coefficient
ρ	rotating frame
R_1	longitudinal or spin-lattice relaxation rate
R_{1f}	longitudinal relaxation rate of H_f in the absence of chemical exchange
R_{1fsat}	longitudinal relaxation rate of H_f as H_r is saturated
R_{1r}	longitudinal relaxation rate of H_r in the absence of chemical exchange
R_2	transverse or spin-spin relaxation rate
rf	radiofrequency
r_i	two-dimensional distance of each particle (i) from the origin
ρ_I, ρ_S	self-relaxation rates of spins I and S
RMS	root-mean-square

σ_{IS}	cross-relaxation rate between spins I and S
T	temperature
T	torque
T_1	longitudinal or spin-lattice relaxation time
t_1	time between 90° rf pulse and first gradient pulse in PGSE diffusion sequence
T_{1f}	spin-lattice relaxation time of H_f in the absence of chemical exchange
T_{1fsat}	longitudinal relaxation time of H_f as H_f is saturated
T_2	transverse or spin-spin relaxation time
t_2	time between second gradient pulse and echo maximum in PGSE diffusion sequence
τ_c	correlation time
t_d	diffusion time
TE	echo time in spin-echo pulse sequence
t_i	time of the i th zero-crossing of the FID
t_{irr}	duration of rf irradiation
t_p	duration of radiofrequency pulse
TR	repetition time
Tr	trace
W	transition probabilities per unit time
W_0	probability of zero-quantum transitions per unit time
W_1	probability of single-quantum transitions per unit time
W_2	probability of double-quantum transitions per unit time
ω_{eff}	precessional frequency of magnetization in rotating frame
WM	white matter
ω_0	Larmor frequency
ω_{rf}	frequency of applied radiofrequency magnetic field
x_ρ, y_ρ, z_ρ	cartesian coordinates in the rotating reference frame
Ψ	spin wave-function
$ \alpha\rangle$	spin state of spin-1/2 particle with $I_z = 1/2$
$ \beta\rangle$	spin state of spin-1/2 particle with $I_z = -1/2$
μ	magnetic moment
μ_x	x component of magnetic moment
μ_y	y component of magnetic moment

μ_z	z component of magnetic moment
$\pi/2_i$	radiofrequency pulse applied along the i th direction in rotating frame that produces a tip angle (Θ) of 90°
π_i	radiofrequency pulse applied along the i th direction in rotating frame that produces a tip angle (Θ) of 180°
Ω	rotational frequency of rotating reference frame
∇	gradient
$\nabla \cdot$	divergence

CHAPTER 1

Preliminary Remarks

Water is the most common liquid on earth and also one of the most unusual. Without the unique and peculiar properties of water, there would be no life, at least as we know it, on earth. For example, if hydrogen bonding did not dominate the properties of water, water would melt at -90°C and boil at -80°C (at 1 atm pressure) (Luck 1974). Water often was thought of as an inert solvent in biological systems. However, it is presently known that water plays an important role in both the structure and function of biomolecules. Deoxyribonucleic acid (DNA), the fundamental molecule of life, is denatured by dehydration (Luck 1974). In addition, water is known to have profound effects on the stability, function, and structure of proteins (Berndt 1993). The same hydrophobic (water-fearing) effect that drives hydrophobic residues to the internal part of proteins is also responsible for the formation of lipid bilayers (i.e. membranes) in aqueous solution.

Despite the obvious importance of water to biological processes, few scientists investigate the properties of water (Franks 1972, Lauger 1988). There are probably two reasons for this lack of enthusiasm for water research. First, water is quite a familiar substance and its properties, despite not being fully understood, often are taken for granted. Second, it is quite difficult to study the contribution of water to life processes.

Nuclear magnetic resonance (NMR) spectroscopy has proven to be a useful technique for the study of solutions, the focus generally being on the solute of interest (e.g. macromolecules) rather than the solvent (e.g. water). However, in magnetic resonance imaging (MRI) of biological tissues, the solvent is of great importance since it is the signal from the protons (i.e. the nucleus of the hydrogen atom) of the water molecule that is mapped to yield an image of the tissue, non-invasively. Therefore, MRI scientists are concerned primarily with the effects of the macromolecules on water (and subsequent water-water interactions) because the macromolecule-water interactions control our ability to distinguish tissue types (e.g. pathological versus normal).

Longitudinal and transverse relaxation (defined in Section 2.1.4) of water protons are the usual sources of tissue contrast in MRI. Even though relaxation is the workhorse of diagnostic MRI, it has proven to be quite *non-specific*. For example, MRI is a useful procedure for identifying multiple sclerosis (MS) lesions in-vivo (Paty 1988). However, using typical relaxation-weighted images, it is difficult to distinguish acute lesions (inflammatory) from chronic lesions (demyelinated, gliotic). The overall goal of this research programme is to show how in-vivo NMR can be made quantitatively sensitive to specific pathological changes, and hence improve the specificity in the diagnostic imaging of white matter disease. Previous graduate students, Ravi Menon, Ph.D., (Menon 1990) and Frances Fenrich, M.Sc., (Fenrich 1992) concentrated their efforts on the relaxation characteristics of water protons in white matter models (to be discussed in Section 2.2.1).

This thesis concentrates on two additional water properties, diffusion and magnetization transfer (MT) (discussed in Sections 2.1.5 and 2.1.6, respectively). Imaging sequences have been developed recently to exploit these water properties and can be utilized for unique MRI contrast or to provide information that complements proton relaxation. In order to understand the dependence of the various tissue water NMR parameters on the physiological microstructure, the diffusion and MT characteristics of water in simplified white matter models will be evaluated. Chapter 2 of this thesis provides some basic NMR theory and details of NMR measurements of diffusion and magnetization transfer. Also, a discussion of the background of water properties pertinent to NMR studies of biological systems (brain and nerve, in particular) is presented in Chapter 2. This allows us to introduce and address the questions that are relevant to this thesis. Chapter 3 deals with the experimental materials and methods. In addition, validation of our measurement techniques using phantoms (known samples) are presented in Chapter 3.

Chapters 4 - 7 include the results and discussions of our experimental measurements on nerve. Chapter 4 is concerned with the determinants of anisotropic water diffusion in nerve (i.e. the preferential mobility of water along the length of the fiber as opposed to across it). The olfactory, trigeminal, and optic nerves of the garfish, the giant axon of the squid, and the sciatic nerve of the frog are used as our white matter models. The role of myelin, microtubules, fast axonal transport, neurofilaments, and local magnetic-susceptibility-induced gradients on anisotropic

diffusion are tested in the aforementioned nerve samples. In addition, we relate the loss of anisotropic diffusion of water in the garfish nerves with structural degradation in-vitro. q -space analysis of the full diffusion curves is presented for normal garfish nerve.

Chapter 5 describes an attempt to measure the diffusion coefficients of water in individual compartments (e.g. axoplasm, myelin, inter-axonal) in normal garfish nerve, using differences in the transverse relaxation of water to isolate compartments. Chapter 6 illustrates the ability of water diffusion to identify degenerated nerve. In particular, a crush or tie of the sciatic nerve of the frog provides a model of Wallerian degeneration. In Chapter 7 we switch gears, leave our discussion of diffusion, and proceed to the measurements of magnetization transfer in the normal myelinated and nonmyelinated nerves of the garfish. The major results of Chapters 4 - 7 are summarized in the concluding chapter (Chapter 8) and future directions of study are suggested.

CHAPTER 2

Theory and Background

Some basic NMR principles, particularly those aspects which are pertinent to this thesis, are presented in the first part of this chapter (2.1). The effects of diffusion and magnetization transfer in NMR will also be evaluated. The second part of the chapter (2.2) is devoted to a background of several NMR relevant properties (transverse and longitudinal relaxation, diffusion, and magnetization transfer) of water in biological systems (brain and nerve, in particular). The questions that this thesis hopes to answer and our proposed solutions are introduced in 2.2.

2.1 Some Aspects of Nuclear Magnetic Resonance

Nuclear magnetic resonance is a diverse and complex research tool. It can probe the structure and dynamics of many gases, liquids, semi-solids (e.g. biological tissues), and solids. There are many useful books which cover some of the basics and applications of NMR (Abragam 1961a, Ernst 1987, Fukushima 1981a, Jardetzky 1981a, and Slichter 1990a). We will cover those basics which are directly pertinent to this thesis.

2.1.1 Spin and Magnetic Moments

A rotating, charged body possesses angular momentum and a magnetic moment, and is expected to interact with an applied magnetic field. According to classical mechanics, the orientation of the angular momentum can take any value and, hence, the rotating body can be in any orientation with respect to the field. However, according to quantum mechanics, the angular momentum of elementary particles, such as protons, neutrons, and electrons, is quantized and, therefore, the rotating body can only possess discrete orientations. In 1921, Stern and Gerlach confirmed the quantum mechanical prediction by experimentally showing that a beam of silver atoms, which have one unpaired electron, splits into two discrete bands when passed through an inhomogeneous magnetic field (Atkins 1986a). The observation of only two bands

conflicted with the quantum mechanical prediction that an angular momentum, l , should yield $2l+1$ orientations, where l is an integer. Hence, it was postulated that they were not observing the orbital angular momentum due to motion of the electron around the nucleus, but rather the motion of the electron about its own axis (supposedly).

The angular momentum associated with this axial motion of the electron (and other elementary particles) is referred to as **spin**. In order to get two bands for the silver atoms, it was necessary to allow the spin quantum number (I) to possess half-integer values. Therefore, a spin of $1/2$ was derived for the electron (i.e. $2I+1 = 2(1/2)+1 = 2$ bands). In a similar manner, it was shown that neutrons and protons are also spin- $1/2$ particles. Since a neutron has no charge, the simple model of a rotating, charged particle (with angular momentum) generating a magnetic moment (μ) is naive. In fact, spin must be a function of the elementary particles that comprise protons and neutrons.

Nevertheless, there is still a relationship between the spin angular momentum and the nuclear magnetic moment. The magnitude of the total spin angular momentum is $\{I(I+1)\}^{1/2} \hbar$ ($\hbar = h/2\pi$ where h is Planck's constant) whereas the component quantized along the field (z -direction) is given by $m_I \hbar$. m_I is the magnetic spin quantum number and is equal to $-I, -I+1, \dots, I-1, I$. The x and y components of the angular momentum are unknown since the uncertainty principle does not allow the exact specification of more than one component. The magnetogyric ratio (γ), which is a constant characteristic for a given nucleus, is equal to the nuclear magnetic moment divided by the maximum value of the z -component of the spin angular momentum, i.e.

$$\gamma = \frac{\mu}{I\hbar} \quad . \quad [2.1]$$

Interestingly enough, magnetic resonance was first developed in 1938 to measure the magnetogyric ratios and magnetic moments of nuclei in molecular beams (Rabi 1938, Rabi 1939).

2.1.2 Interaction of Protons with a Static Homogeneous Magnetic Field

2.1.2.1 Single Spin

The magnetic moment ($\boldsymbol{\mu}$) of a spin and an external static magnetic field oriented along the z-direction, $\mathbf{B}_0$, have a Hamiltonian ($\mathcal{H}$) describing their interaction energy,

$$\mathcal{H} = - \boldsymbol{\mu} \cdot \mathbf{B}_0 = - \gamma \hbar B_0 I_z \quad . \quad [2.2]$$

By substituting this Hamiltonian into the time-independent Schrödinger equation

$$\mathcal{H}\Psi = E \Psi \quad [2.3]$$

and using the orthonormal spin wave-functions $\Psi = |\alpha\rangle$ (with $I_z = 1/2$) or $|\beta\rangle$ (with $I_z = -1/2$) for a proton, the energies (E) of the $|\alpha\rangle$ and $|\beta\rangle$ states are $-\gamma \hbar B_0/2$ and $\gamma \hbar B_0/2$, respectively. The spin aligned "parallel" to $\mathbf{B}_0$, $|\alpha\rangle$, is of lower energy than the "anti-parallel" spin, $|\beta\rangle$. Hence, the introduction of a spin-1/2 particle into a magnetic field generates a very small, but measurable, energy difference (ΔE) of $\gamma \hbar B_0$ between its two spin states. Hence, in analogy to optical spectroscopies, an energy $h\nu_0$ ($\nu_0 = \omega_0/2\pi$) is required to "excite" an $|\alpha\rangle$ spin to the $|\beta\rangle$ state.

One can also interrogate the interaction of $\boldsymbol{\mu}$ and $\mathbf{B}_0$ with classical mechanics. Classically, a nuclear magnetic moment ($\boldsymbol{\mu}$) in a field, $\mathbf{B}_0$, experiences a torque, $\mathbf{T}$, given by

$$\mathbf{T} = \boldsymbol{\mu} \times \mathbf{B}_0 \quad . \quad [2.4]$$

Since torque is the rate of change of angular momentum (I), $\mathbf{T}$ can be replaced with $\frac{dI}{dt}$

$$= \frac{d(\boldsymbol{\mu}/\gamma)}{dt},$$

$$\frac{d\boldsymbol{\mu}}{dt} = \gamma \boldsymbol{\mu} \times \mathbf{B}_0 \quad . \quad [2.5]$$

Evaluating the cross-product and replacing γB_0 with a frequency ω_0 yields

$$\frac{d\mu_x}{dt} = \omega_0 \mu_y, \quad \frac{d\mu_y}{dt} = -\omega_0 \mu_x, \quad \frac{d\mu_z}{dt} = 0 \quad . \quad [2.6]$$

Let $\mu_x(0)$, $\mu_y(0)$, and $\mu_z(0)$ be the Cartesian components of the magnetic moment at $t = 0$. Combining μ_x and μ_y as a complex pair, $\mu_{xy} = \mu_x + i \mu_y$, where $i = (-1)^{1/2}$, Eqn. 2.6 becomes

$$\frac{d(\mu_x + i\mu_y)}{dt} = \frac{d\mu_{xy}}{dt} = -i \omega_0 \mu_{xy}, \quad \frac{d\mu_z}{dt} = 0 \quad . \quad [2.7]$$

Solving these differential equations yields the following solutions,

$$\begin{aligned} \mu_x &= \mu_x(0) \cos(\omega_0 t) + \mu_y(0) \sin(\omega_0 t) \\ \mu_y &= -\mu_x(0) \sin(\omega_0 t) + \mu_y(0) \cos(\omega_0 t) \\ \mu_z &= \mu_z(0) = \text{constant} \quad . \end{aligned} \quad [2.8]$$

Therefore, in the absence of external interactions, $\boldsymbol{\mu}$ undergoes undamped steady precession counter-clockwise about $\mathbf{B}_0$ with a frequency known as the Larmor frequency,

$$\omega_0 = \gamma B_0 \quad . \quad [2.9]$$

The precessional frequency of protons (with $\gamma = 2.6752 \times 10^8 \text{ rad T}^{-1} \text{ s}^{-1}$) at a field strength of 2.35 T is 100 MHz (i.e. in the radiofrequency range). Historically, in 1938, Rabi used the measurement of the precessional frequency of various nuclei in a static magnetic field of known strength to calculate γ and subsequently the magnetic moment of nuclei (Rabi 1938, Rabi 1939).

2.1.2.2 Ensemble of Spins

The magnetic moment of a single spin cannot be detected. Fortunately, a real sample usually contains numerous spins (e.g. $\sim 7 \times 10^{22}$ protons per mL of water). Spontaneous emission is negligible in the radiofrequency range. Thus, upon

introduction into a magnetic field, changes in spin states must be induced by stimulated emission or stimulated absorption. In other words, the spins must be connected to some energy source or sink in order to rearrange their occupancies of the available energy levels. This follows from the conservation of energy. In fact, the spins are connected with the thermal motion of the molecules.

The tendency of the nuclei to occupy the lowest energy level, $|\alpha\rangle$, is opposed by thermal motion which tends to distribute the spin population amongst the energy levels. Since the lattice (thermal motions of rotation and translation) is in thermal equilibrium and since the populations of the energy levels of the lattice follows a Boltzmann distribution, the spin states will also be driven into a Boltzmann distribution. The specific heat of the lattice is assumed to be infinite and hence, the lattice probabilities are not modified by the energy transfer between the lattice and the spins. The proportion of $|\alpha\rangle$ spins, $P_{|\alpha\rangle}$, and $|\beta\rangle$ spins, $P_{|\beta\rangle}$, is given by

$$\frac{P_{|\alpha\rangle}}{P_{|\beta\rangle}} = \exp(\Delta E / kT) = \exp(\gamma \hbar B_0 / kT) \quad [2.10]$$

where k is the Boltzmann constant and T is the temperature in Kelvin. For protons at a temperature of 293 K and $B_0 = 2.35$ T, $P_{|\alpha\rangle}/P_{|\beta\rangle}$ is 1.000016 (i.e. for every 1,000,000 spins in the $|\beta\rangle$ state, there are 1,000,016 in the $|\alpha\rangle$ state). This small excess of $|\alpha\rangle$ spins leads to a net magnetic moment ($\mathbf{M}$) aligned along $\mathbf{B}_0$ (in the z -direction). At thermal equilibrium, the phases of the individual nuclear magnetic moments are random. Therefore, there are no transverse (x and y) components of net magnetization at equilibrium. It would be difficult to observe $\mathbf{M}$ directly. A method of detecting the net magnetization using a time-varying magnetic field is discussed in the next section.

2.1.3 Interaction of Protons with Both a Static and Time-Varying Magnetic Field

It was recognized early on that a reorientation of the nuclear spin and magnetic moment, with respect to the static magnetic field ($\mathbf{B}_0$), can occur if a small magnetic field ($\mathbf{B}_1$) oscillating at the Larmor frequency (ω_0) is applied transverse to $\mathbf{B}_0$ (Rabi 1938). In 1946, Bloch at Stanford and Purcell at Harvard, independently, were able to use this principle to measure, for the first time, magnetic resonance in "matter of

ordinary density" such as water and paraffin (Bloch 1946a, Purcell 1946). Rabi noted that this reorientation process is more accurately described quantum mechanically by a mixture (or linear combination) of the spin states. We will not pursue this description. Instead, we will focus on Felix Bloch's approach of describing the motion of the classical magnetization vectors in a field (Bloch 1946b), since this formalism applies to fluid samples in which the spins are assumed to be isolated and uncoupled.

Classically, a magnetic moment experiences a torque in a magnetic field (Eqn. 2.4). Analogous to Eqn. 2.5, the rate of change of the net magnetization ($\mathbf{M}$) of an ensemble of spins experiencing a static magnetic field ($\mathbf{B}_0$) and a time-varying magnetic field $\{\mathbf{B}_1(t)\}$ with respect to time, in the laboratory frame, is

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} = \mathbf{M} \times \gamma \{\mathbf{B}_0 + \mathbf{B}_1(t)\} \quad [2.11]$$

where $\times$ refers to a cross-product. Generally, the radiofrequency field (rf) we apply is linearly polarized along the x-axis, with $\mathbf{B}_0$ defining the z-axis (usual convention). A linearly polarized field $2B_1\cos(\omega_{rf}t)$ can be considered as a superposition of two B_1 fields counter-rotating with angular velocities $\pm \omega_{rf}$. Only the component of rf precessing in the same direction as $\boldsymbol{\mu}$ (i.e. $-\omega_{rf}$) will interact with $\boldsymbol{\mu}$. The time dependent magnetic field is therefore

$$\mathbf{B}_1(t) = B_1 \{\cos(\omega_{rf}t) \mathbf{i} - \sin(\omega_{rf}t) \mathbf{j}\} \quad [2.12]$$

and the static magnetic field is

$$\mathbf{B}_0 = B_0 \mathbf{k} \quad [2.13]$$

where $\mathbf{i}$, $\mathbf{j}$, and $\mathbf{k}$ are unit vectors along the x, y, and z axes, respectively. Now, evaluating $\mathbf{M} \times \mathbf{B}$ from the following determinant

$$\begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ M_x & M_y & M_z \\ B_1\cos(\omega_{rf}t) & -B_1\sin(\omega_{rf}t) & B_0 \end{vmatrix} \quad [2.14]$$

and substituting into Eqn. 2.11 yields the following Bloch equations in the lab frame (neglecting relaxation for the time being),

$$\begin{aligned}\frac{dM_x}{dt} &= \gamma \{M_y B_0 + M_z B_1 \sin(\omega_{rf} t)\} \\ \frac{dM_y}{dt} &= \gamma \{M_z B_1 \cos(\omega_{rf} t) - M_x B_0\} \\ \frac{dM_z}{dt} &= -\gamma \{M_x B_1 \sin(\omega_{rf} t) + M_y B_1 \cos(\omega_{rf} t)\}\end{aligned}\quad [2.15]$$

However, it will be easier to visualize the effects of the rf field, $\mathbf{B}_1(t)$, if we can neglect the effect of precession about $\mathbf{B}_0$. We therefore introduce a reference frame that is rotating about the z-axis at a frequency Ω and use $\partial/\partial t$ to denote a time derivative of a quantity observed from this rotating frame. It follows that Eqn. 2.11 transforms to (Slichter 1990b)

$$\begin{aligned}\frac{\partial \mathbf{M}}{\partial t} &= \mathbf{M} \times \{(\Omega + \gamma B_0) \mathbf{k} + \gamma B_1 \mathbf{i}\} \\ &= \mathbf{M} \times \gamma \{(B_0 + \Omega/\gamma) \mathbf{k} + B_1 \mathbf{i}\}\end{aligned}\quad [2.16]$$

We can introduce an effective magnetic field in the rotating frame,

$$\mathbf{B}_{eff} = (B_0 + \Omega/\gamma) \mathbf{k} + B_1 \mathbf{i} \quad [2.17]$$

and Eqn. 2.16 becomes

$$\frac{\partial \mathbf{M}}{\partial t} = \mathbf{M} \times \gamma \mathbf{B}_{eff} \quad [2.18]$$

Therefore, in a completely analogous manner to our discussion of $\boldsymbol{\mu}$ and $\mathbf{B}_0$ in Section 2.1.2.1, $\mathbf{M}$ precesses about the effective magnetic field with a counter-clockwise frequency (ω_{eff}) (Fig. 2.1a),

$$\omega_{eff} = \gamma B_{eff} \quad [2.19]$$

The cartesian coordinates in the rotating reference frame are given by x_ρ , y_ρ , and z_ρ (note: ρ indicates the rotating reference frame).

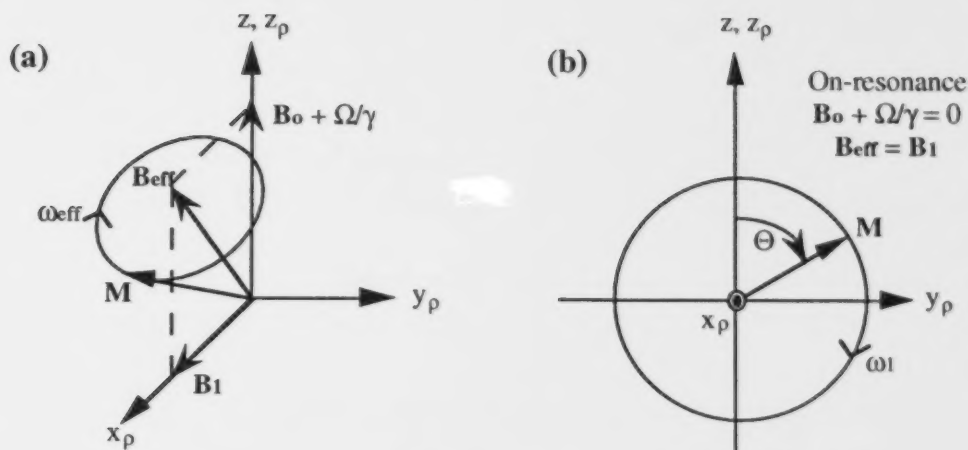


Figure 2.1 The rotating frame representation of the motion of the net magnetization $\mathbf{M}$ in a field $\mathbf{B}_{\text{eff}}$. (a) The general case in which the reference frame is rotating at a frequency Ω . $\mathbf{M}$ rotates counter-clockwise at a frequency $\omega_{\text{eff}} (= \gamma B_{\text{eff}})$ around $\mathbf{B}_{\text{eff}}$. (b) When $\Omega = -\omega_0$, $\mathbf{B}_{\text{eff}} = B_1 \mathbf{i}$, and $\mathbf{M}$ precesses in the $y_\rho z_\rho$ plane at a frequency $\omega_1 (= \gamma B_1)$. For an rf pulse of duration t_p , $\mathbf{M}$ precesses through an angle $\Theta = \omega_1 t_p$.

$B_0 + \Omega/\gamma$ is a measure of the off-resonance between the applied rf field and the magnetization of the nuclei of interest. When the angular frequency of the reference frame is set equal to the resonance frequency, $-\omega_0$, then the contribution of B_0 along the z_ρ axis is nullified (i.e. $B_0 - \omega_0/\gamma = 0$), leaving only a static B_1 along the x_ρ axis. The cross product of Eqn. 2.18 already indicates that the change in $\mathbf{M}$ will be perpendicular to $\mathbf{M}$ and $\mathbf{B}_1$. In this case, $\omega_{\text{eff}} = \gamma B_1$ and $\mathbf{M}$ precesses in the $y_\rho z_\rho$ plane (Fig. 2.1b). Applying an rf pulse for a time t_p will result in an angular displacement Θ of $\mathbf{M}$ from its equilibrium position along the z -axis, namely

$$\Theta = \omega_{\text{eff}} t = \gamma B_1 t_p \quad . \quad [2.20]$$

By choosing an appropriate pulse length for the available rf strength, the net magnetization ($\mathbf{M}$) can be tipped by $\pi/2$ rad (or 90°) and placed along y_ρ in the plane transverse to $\mathbf{B}_0$. Even though $\mathbf{M}(y_\rho)$ is static in the rotating frame, it is actually

precessing at ω_0 in the laboratory frame. This precessing moment induces an electromotive force (EMF) in a coil (usually tuned to ω_0 for maximum efficiency) which generates a current if we have a closed loop. Hence, we are able to detect the original equilibrium magnetization by observing the consequences of driving it away from equilibrium.

In order to achieve a 90° tip angle for protons, a rf pulse applied for $10\ \mu\text{s}$ requires a B_1 strength of $587\ \mu\text{T}$. It is interesting that this B_1 strength is only 0.025% of the original B_0 strength ($B_0 = 2.35\ \text{T}$), yet it is able to tip the net magnetization from the overwhelming B_0 direction. The important point is that B_1 is time-varying and introduces a phase coherence in the precession of the individual magnetic moments about B_0 . If B_1 was static (in the lab frame), B_0 and B_1 would add vectorially and the effective field would deviate by a measly 0.014° from the original B_0 direction. Hence, a *static* B_1 would have little effect on M . However, the torque on M is non-negligible if B_1 rotates with the Larmor frequency.

If B_1 and the magnetization do not rotate with the same frequency (i.e. $\Omega \neq -\omega_0$), the torque can quickly get out of phase, and the *average* effect over many Larmor precession frequencies will be small. Also, $B_0 + \Omega/\gamma$ along the z-axis would no longer cancel and consequently, M would not precess in the $y_\rho z_\rho$ plane. Hence, the maximum signal (maximum M along y_ρ after 90° pulse) or an inversion of the magnetization to the $-z$ axis (180° pulse) cannot be obtained if the rf is off-resonance. However, if $B_1 \gg B_0 + \Omega/\gamma$, then $B_{\text{eff}} \sim B_1 \hat{i}$, and the off-resonance effects will be limited in severity.

The Bloch equations (Eqn. 2.15) are clearly a non-linear system of equations, and hence the response of the nuclear magnetic moments to rf is non-linear. For example, doubling the length or amplitude of a 90° pulse does not yield twice the detectable magnetization in the transverse plane. In fact, doubling the 90° pulse places M along the $-z$ axis and no transverse components are generated.

2.1.4 Longitudinal and Transverse Relaxation

As we mentioned in Section 2.1.2.2, coupling of the nuclei in a sample to the lattice allows the nuclear spins to attain thermal equilibrium amongst the nuclear energy levels, and hence, generate a net magnetization along $\mathbf{B}_0$. How does this coupling occur and how long does it take to achieve equilibrium? In addition, once the net magnetization is placed into the transverse plane, how is the transverse magnetization lost with a concomitant return of the spin system to thermal equilibrium? In order to answer these questions, the internal interactions of the nuclei with the lattice and with each other must be considered, and the concept of nuclear relaxation has to be introduced (Bloch 1946b).

Two empirical requirements need to be satisfied at *thermal equilibrium*,

1) the magnetization parallel (longitudinal) to $\mathbf{B}_0$ (i.e. M_z) has a finite value M_0 , i.e. $M_z = M_0$, and

2) the magnetization transverse to $\mathbf{B}_0$ (i.e. M_{xy}) is zero.

Bloch assumed that the approach to M_0 and the decay of M_{xy} were separable, and could individually be described as first order rate processes (i.e. exponential approaches to equilibrium), driven by the offset from equilibrium. This appears to be a valid assumption for mobile environments, such as liquids, but not for solids. The rate of change of M_z and M_{xy} with respect to time is given by

$$\frac{dM_z}{dt} = R_1 (M_0 - M_z) \quad [2.21]$$

$$\frac{dM_{xy}}{dt} = R_2 (0 - M_{xy}) = - R_2 M_{xy} \quad [2.22]$$

where R_1 and R_2 are first-order rate constants. Assume that the magnetization is in the transverse plane ($M_{xy} = M_0$ and $M_z = 0$) at $t = 0$. Using this initial boundary condition, the solutions of Eqns. 2.21 and 2.22 are

$$M_z(t) = M_0 \{ 1 - \exp(-t / T_1) \} \quad [2.23]$$

$$M_{xy}(t) = M_0 \exp(-t / T_2) \quad [2.24]$$

where $T_1 (= 1/R_1)$ is the longitudinal or spin-lattice relaxation time and $T_2 (= 1/R_2)$ is the transverse or spin-spin relaxation time. T_1 represents the time required to achieve $(1 - 1/e)$ (i.e. ~63%) of the original longitudinal magnetization and T_2 is the time required for the transverse magnetization to decrease to $1/e$ (i.e. ~37%) of its original value. Combining Eqns. 2.21 and 2.22 with Eqn. 2.11, which only takes into account the effects of the external fields (i.e. $\mathbf{B}_0$ and $\mathbf{B}_1(t)$), yields the basic Bloch equation

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} + \frac{M_0 - M_z}{T_1} \mathbf{k} \quad [2.25]$$

The detailed analysis of relaxation is discussed in greater detail elsewhere (Bloembergen 1948, McConnell 1987, Fukushima 1981b, Jardetzky 1981b). Therefore, only some of the salient features of relaxation are presented. The mechanism of relaxation is similar to the interaction of an applied time-varying rf field $\{\mathbf{B}_1(t)\}$ with the nuclear magnetic moments. However, for relaxation, the time-varying magnetic fields felt at the nucleus are *randomly* fluctuating. In diamagnetic liquids (such as water), the dipole-dipole interaction between nuclear magnetic moments is the dominant source of the randomly fluctuating fields. The time-dependent nature of the fields is due to the molecular motion (translations, rotations) and natural flip rate of the spins. The random motion of the molecules is characterized by a correlation time, τ_c . τ_c can be considered to be the time at which there is no clear relationship between the state of the system at time t and $t + \tau_c$. Rapid fluctuations yield small correlation times, whereas slower fluctuations yield a larger τ_c . For example, the τ_c of pure water and macromolecules are typically $\sim 10^{-12}$ s and 10^{-8} s, respectively. The efficiency of relaxation is linked to the correlation time (Bloembergen 1948), and hence, the utility of relaxation time measurements lies in their sensitivity to the thermal motions of molecules.

Longitudinal (spin-lattice) relaxation involves the transfer of energy from the spin system to the lattice (the thermal motions) in order to re-establish thermal equilibrium of the nuclear spin system. Those magnetic field fluctuations that have Fourier components at ω_0 and $2\omega_0$ (ω_0 is the Larmor frequency) are capable of inducing energy transfers between the spin system and the lattice. A good explanation of why fluctuations with frequency components at $2\omega_0$ can induce relaxation is presented on page 697 of Bloembergen et al. (1948). Consequently, the spins will

lose energy to the lattice via transitions from the $|\beta\rangle$ to $|\alpha\rangle$ state. The Boltzmann distribution of the spin system will be re-established with a concomitant return of the net magnetization along $\mathbf{B}_0$. Relaxation is most efficient (and T_1 the smallest) when the motions of the molecules (or parts thereof) "tune" in to the resonance frequency of the spins.

Transverse (spin-spin) relaxation does not involve the net transfer of energy to the lattice. Rather, the nuclear precessions exchange energy with one another, the net effect being a reduction of the magnetization in the transverse plane. Fourier components of molecular motions at "zero", ω_0 , and $2\omega_0$ frequencies affect T_2 . The ever-changing z-components of the local fields $\{(B_z)_{loc}\}$ of neighbouring nuclei can add or subtract to $\mathbf{B}_0$ and cause slightly different Larmor precessional frequencies for each nucleus. This results in irreversible dephasing of the transverse magnetization. Also, for identical nuclei, the simultaneous flip-flop of two anti-parallel spins limits the life-time of a spin in a given state and reduces M_{xy} . The $(B_z)_{loc}$ and the flip-flop mechanism correspond to the "zero" frequency interactions, which are not present in the T_1 process. The longitudinal magnetization (M_z) is unaffected by $(B_z)_{loc}$ since $(B_z)_{loc}$ is parallel to M_z (the fluctuating field must be perpendicular to M in order to have an effect). The flip-flop mechanism will not affect T_1 since the energy of the spin-system is conserved. Nevertheless, T_2 is closely related to T_1 because we can never have $T_1 < T_2$. In other words, there must be no residual transverse magnetization if the system has already reached thermal equilibrium. Therefore, the ω_0 and $2\omega_0$ frequency components that are involved in longitudinal relaxation also influence transverse relaxation.

If the molecular fluctuations are quite rapid and τ_c is quite short ($1/\tau_c \gg \omega_0$), as in pure water (i.e. $1/10^{-12} \gg 10^8$ Hz), the "zero" frequency components average out and their contributions to T_2 will be negligible. In this case, $T_1 \equiv T_2$ and is of the order of a few seconds for pure water. On the other hand, if the molecular fluctuations are quite slow and τ_c is quite long ($1/\tau_c \ll \omega_0$), as in water adsorbed to a slowly tumbling macromolecule (i.e. $1/10^{-8} \ll 10^8$ Hz), then the "zero" frequency terms dominate and $T_2 < T_1$. The slower motions of water in biological systems makes the transverse relaxation more efficient than the longitudinal relaxation.

The Inversion Recovery (IR) pulse sequence is typically used to measure T_1 (Carr 1954). The IR pulse sequence is

$$180 - \tau - 90 - \text{AQ}$$

where 180 and 90 refer to 180° and 90° rf pulses, respectively, τ is a variable time interval, and AQ represents the acquisition interval of the free-induction-decay (FID) of the magnetization. The 180° pulse places the original net magnetization, M_0 , along the $-z$ axis. As the time τ is incremented, $-M_z$ becomes less negative, passes through zero, and increases to M_0 when τ is large. The 90° pulse samples the magnetization that remains along the z axis. For a one-component system, a plot of $\ln\{[M_0 - M(\tau)]/M_0\}$ versus τ yields a slope of $-1/T_1$ (see Eqn. 2.23 except that the exponential term is multiplied by a factor of 2 for IR sequence).

The Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence is used to measure T_2 (Carr 1954, Meiboom 1958). The CPMG pulse sequence is

$$90_x - \tau - (180_y - 2\tau)_n$$

where the acquisition of the n spin-echoes occurs during the middle of the period 2τ . The subscripts x and y describe the relative phases of the 90° and 180° rf pulses in the rotating frame. The 90 degree phase-shift between the 90° and 180° rf pulses is used to minimize the errors associated with imperfect 180° rf pulses on the even-order echoes. The 180° rf pulses refocus the loss of transverse magnetization due to field inhomogeneities in the static magnetic field and local inhomogeneities associated with differences in magnetic susceptibility (i.e. proportionality constant between the induced magnetization and the magnetic field that induced it) between distinct substances within the sample. This technique allows, therefore, the measurement of the intrinsic T_2 of the sample of interest. The time 2τ is kept short in order to minimize the irreversible dephasing and loss of transverse magnetization due to diffusion in inhomogeneous magnetic fields (to be discussed further in Section 2.1.5.2). For a one-component system, a plot of $\ln\{M(2\tau n)/M_0\}$ versus the time $2\tau n$ yields a slope of $-1/T_2$ (see Eqn. 2.24). In some cases, the decay of the transverse magnetization is multi-exponential (i.e. the sum of several exponentials) and curve-fitting procedures are required to extract the individual T_2 values and amplitudes of each exponential.

2.1.5 Measurements of Diffusion by NMR

Excellent reviews concerning the measurements of diffusion by NMR are available in the literature (Reeves 1975, Stilbs 1987, Karger 1988a, Callaghan 1991a). We will discuss the basics of diffusion before discussing the link between diffusion and NMR.

2.1.5.1 Basics of Diffusion

Diffusion involves the transfer of molecules from one part of a system to another via the random thermal motions (Brownian motion) of molecules. Often, the time-dependence of the change in position of a solute in a solvent is investigated. Assume we have a thin sheet of solute initially at $x = 0$, for example (Fig. 2.2). The driving force for diffusion is the rate of change of concentration ($\partial c / \partial x$) along the x -direction (i.e. perpendicular to the initial plane of particles). The rate of transfer of molecules through a unit cross-sectional area is given by

$$\frac{\partial m}{\partial t} = -D \frac{\partial c}{\partial x} \quad [2.26]$$

where m is the number of moles of material crossing a unit area perpendicular to the x -axis, c is the concentration (moles per unit volume) of the diffusing substance, and D is the proportionality constant called the **diffusion coefficient** (Barrow 1979a). Eqn. 2.26 is Fick's first law of diffusion.

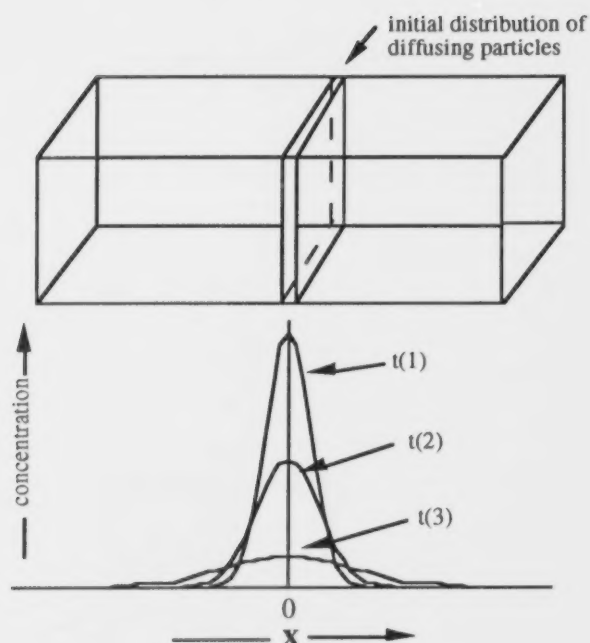


Figure 2.2 The displacement profiles expected after an initial plane of particles undergoes diffusion for durations of $t(1) < t(2) < t(3)$.

Next, we wish to evaluate the effect of diffusion on the concentration in a given volume by determining the net rate at which substances accumulate in that volume. The rate of accumulation is given by the difference between the rate of entry at x and the rate of leaving at $x+\Delta x$ (assuming a unit area, the volume equals the slice thickness Δx),

$$\text{rate of accumulation} = -D \frac{\partial c}{\partial x} - \left\{ -D \left[\frac{\partial c}{\partial x} + \frac{\partial}{\partial x} \left(\frac{\partial c}{\partial x} \right) \Delta x \right] \right\} \quad [2.27]$$

Since concentration is the number of particles per unit volume, the rate of change of concentration in one-dimension (x) is given by the rate of accumulation divided by the volume element (Δx). Therefore,

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad [2.28a]$$

or generally for higher dimensions of diffusion,

$$\frac{\partial c}{\partial t} = \nabla \cdot D \cdot \nabla c \quad [2.28b]$$

where ∇ and $\nabla \cdot$ represent the gradient and divergence, respectively. Eqn. 2.28 is known as Fick's second law of diffusion.

If there are N particles in our initial plane ($x = 0$) at $t = 0$, the solution of Eqn. 2.28a is (Barrow 1979)

$$c = \frac{N}{2(\pi Dt)^{1/2}} \exp(-x^2 / 4Dt) \quad [2.29]$$

The statistical view of diffusion in which the particles jump in a series of small steps yields an equation similar to Eqn. 2.29 (Atkins 1986b). Therefore, the particles migrate from their original positions over time, their final displacements described by a Gaussian distribution centered about their initial starting position (Fig. 2.2). It follows that the one-dimensional root-mean-square displacement (λ_1) of the particles is

$$\lambda_1 = \sqrt{2Dt} \quad [2.30]$$

where t is the duration of diffusion. 68% of the particles remain within a distance λ_1 from the origin. The root-mean-square displacements in two (λ_2) and three (λ_3) dimensions are $\sqrt{4Dt}$ and $\sqrt{6Dt}$, respectively. The important point is that λ increases as the square root of the diffusion time.

A commonly cited example of diffusion is that of sucrose (i.e. sugar) in water. At 25°C, sucrose has $D = 0.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Barrow 1979). The λ_1 of sucrose diffusing in water for 1 hour is 1.7 mm. Therefore, this example demonstrates that diffusion is an extremely slow process. D and λ are decreased to a further extent in solutions with large asymmetrical molecules, strong inter-molecular forces, or restrictive boundaries.

In general, the solute and solvent consist of different molecular species. However, when the solute and solvent are identical (e.g. pure water), the process of diffusion is called self-diffusion. A gradient can be introduced in order to measure the self-diffusion coefficients from the profiles of the molecular displacements over time. Typically, gradients due to radioactive tracers, or, in NMR, the phases of the nuclear magnetic moments are used to label the molecules (to be discussed in greater detail in the following portions of Section 2.1.5) and measure diffusion. The implicit assumption is that the labeling does not markedly affect the rate of self-diffusion.

The molecular diffusion of pure water has been extensively studied and will be described briefly since it is directly pertinent to this thesis. Measurements of $D(\text{H}_2\text{O})$ by oxygen (^{18}O), deuterium (^2H), and tritium (^3H) tracers, as well as by NMR measurements, are very similar (Trappeniers 1965). The agreement between these various measurement techniques suggests that proton exchange does not significantly contribute to the NMR measurements of diffusion. The data obtained by Mills for water diffusion are used as our gold standard (Mills 1973). The diffusion coefficient (D) is a sensitive function of temperature (T) and follows the Arrhenius equation

$$D = D_0 \exp(-E_a / kT) \quad [2.31]$$

where E_a is the activation energy (in Joules) for translational diffusion, k is the Boltzmann constant, and D_0 is the pre-exponential factor. The activation energy for pure water is ~ 19 kJ/mol and corresponds to the energy required to break approximately two hydrogen bonds (Eisenberg 1969). The activation energies for cellular water (Chang 1973) and water in rat brain (Hasegawa 1994) are about the same as for pure water. A plot of $\log(D)$ versus $1/T$ (T in Kelvin) should yield a straight line if Eqn. 2.31 is obeyed. The self-diffusion coefficient of pure water as a function of $1/T$ are illustrated in Fig. 2.3. These data were used to predict the D values expected for pure water at the temperatures of our NMR measurements of diffusion. The expected diffusion coefficients for pure water at 20 °C (room temperature) and 37 °C (body temperature) are $1.99 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $3.04 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, respectively.

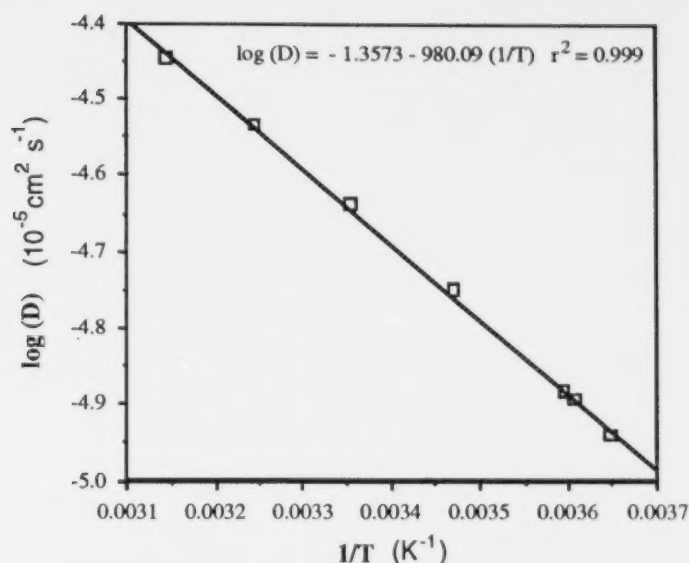


Figure 2.3 The log of the self-diffusion coefficient of pure water as a function of the inverse of the absolute temperature (data from Mills 1973).

2.1.5.2 Diffusion in an Inhomogeneous Static Magnetic Field

The effect of diffusion on transverse magnetization was realized in the first paper on spin-echoes (Hahn 1950). The pulse sequence commonly used to generate a spin-echo is

$$90 - \tau_1 - 180 - \tau_2 - \text{AQ} \quad ,$$

as proposed by Carr and Purcell (Carr 1954) where τ_1 and τ_2 represent equivalent time intervals (i.e. $\tau_1 = \tau_2$) and AQ represents the acquisition period of the spin-echo. We consider the case where the 90° and 180° rf pulses are phase shifted by 90° (i.e. 90_x and 180_y). The 90_x pulse places the net magnetization along the y_ρ axis in the transverse plane. Now, assume that the net magnetization can be described by the sum of individual macroscopic magnetic moment vectors (called **isochromats**) distributed throughout the sample. All of the isochromats precess with identical frequencies (stationary in ρ frame if on-resonance) if the static magnetic field ($\mathbf{B}_0$) is homogeneous. However, if there are inhomogeneities in $\mathbf{B}_0$ over the sample volume,

the individual isochromats experience different magnetic fields and hence precess at slightly different frequencies. As time progresses, the isochromats dephase about the y_ρ axis, with a concomitant decrease in net transverse magnetization. Say that an isochromat, i , has precessed over an angle Φ_i from the y_ρ axis during τ_1 . A 180° pulse applied at τ_1 keeps the i th isochromat in the transverse plane, but places it at an angle $-\Phi_i$ from the y_ρ axis. The isochromat continues to precess in the same direction as it was prior to the application of the 180° rf pulse. Since the isochromat experiences the same field as it did during τ_1 , it precesses Φ_i over the time τ_2 (only if $\tau_1 = \tau_2$). Consequently, the isochromats regain phase coherence and refocus along y_ρ (Fig. 2.4). The transverse magnetization reaches a maximum at $(\tau_1 + \tau_2)$ and a *spin-echo* is formed. However, the refocussed transverse magnetization (i.e. spin-echo) is reduced from its value immediately after the 90° rf pulse because of irreversible transverse relaxation (see Section 2.1.4).

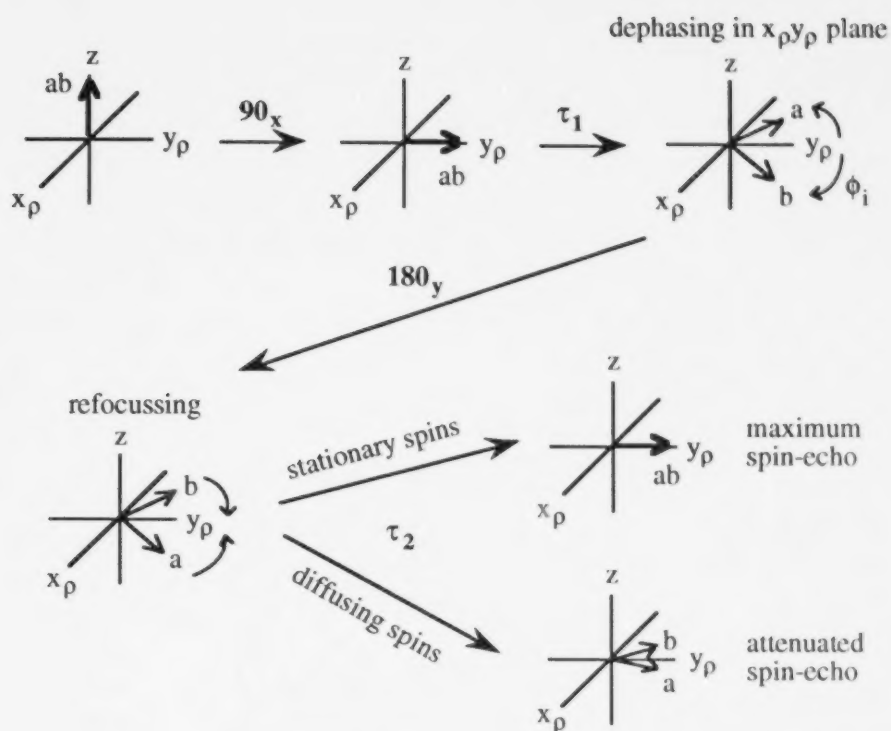


Figure 2.4 The rotating frame response to a spin-echo pulse sequence of two isochromats (a and b) in an inhomogeneous static field. If there is no motion of the spins, a and b fully refocus along y_ρ and yield a maximum spin-echo amplitude. However, if the spins are displaced, a and b do not refocus perfectly along y_ρ (the rf receiver axis), and hence, the spin-echo amplitude is reduced.

Maximum refocussing of the transverse magnetization along y_ρ occurs if the precessional frequencies are constant during τ_1 and τ_2 . This is only true when the isochromats are stationary. If the spatial position of the isochromats is altered, by diffusion for example, the precessional frequencies of the isochromats vary throughout the duration of the pulse sequence. Therefore, the magnetization vectors do not properly refocus along y_ρ (Fig. 2.4). Hence, diffusion of the molecules in an inhomogeneous magnetic field acts to decrease the degree of refocussing of the transverse magnetization. Faster diffusion of the spins and greater spatial inhomogeneities of the static field enhance the attenuation of the spin-echo. The molecules are "labeled" by the relative directions of their nuclear magnetic moments in the transverse plane (i.e. their angular displacement from an arbitrary origin is referred

to as the **phase**). Such a label is quite innocuous and would be expected to have a negligible effect on the diffusion process. Next, we wish to obtain a quantitative relationship between the spin-echo attenuation, the NMR parameters, and the degree of diffusion.

The effects of diffusion on the spin-echo amplitude have been calculated using random walk methods (Carr 1954). We, however, will pursue the macroscopic magnetization treatment of Torrey (Torrey 1956). The effects of diffusion on the rate of change of magnetization with respect to time can be taken into account by adding Fick's second law of diffusion (Eqn. 2.28) to the Bloch equation (Eqn. 2.25). If diffusion is isotropic, then $\nabla \cdot \mathbf{D} \cdot \nabla$ can be written as $D\nabla^2$ (where $\nabla = \partial/\partial \mathbf{r}$). Therefore, the modified Bloch equation is

$$\frac{\partial \mathbf{M}(\mathbf{r}, t)}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}(\mathbf{r}, t) - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} - \frac{M_z - M_0}{T_1} \mathbf{k} + D \nabla^2 \mathbf{M} \quad [2.32]$$

where $\mathbf{M}$ and $\mathbf{B}$ are functions of both space ($\mathbf{r}$) and time (t). Assume that the inhomogeneities in the magnetic field are linear and time-independent across the sample. It follows that the static magnetic field is given by

$$B_z(\mathbf{r}, t) = B_0 + (\mathbf{G} \cdot \mathbf{r}) \quad [2.33]$$

where $\mathbf{G}$ is the gradient strength of the magnetic field (i.e. $\mathbf{G} = \partial B_z / \partial \mathbf{r} = \mathbf{i} \partial B_z / \partial x + \mathbf{j} \partial B_z / \partial y + \mathbf{k} \partial B_z / \partial z$) and $\mathbf{r}$ represents the displacement from an arbitrary origin. Notice that we are only interested in B_z since B_x and B_y are approximately zero in practise. The net magnetic field affects the precessional frequency (and thus the attainable phase) of the components of the transverse magnetization.

In the absence of an rf field, Eqn. 2.32 yields the rate of change of the transverse magnetization components as,

$$\frac{\partial M_x}{\partial t} = \gamma M_y B_0 + \gamma M_y \mathbf{G} \cdot \mathbf{r} - \frac{M_x}{T_2} + D \nabla^2 M_x \quad [2.34a]$$

$$\frac{\partial M_y}{\partial t} = -\gamma M_x B_0 - \gamma M_x \mathbf{G} \cdot \mathbf{r} - \frac{M_y}{T_2} + D \nabla^2 M_y \quad [2.34b]$$

The transverse magnetization (M_+) is given by $M_+ = M_x + i M_y$, and Eqns. 2.34a and 2.34b reduce to

$$\frac{\partial M_+}{\partial t} = i \omega_0 M_+ - \frac{M_+}{T_2} - i \gamma (\mathbf{G} \cdot \mathbf{r}) M_+ + D \nabla^2 M_+ \quad [2.35]$$

The precession and relaxation can be isolated by defining $M_+(\mathbf{r}, t)$ as

$$M_+(\mathbf{r}, t) = m_+(\mathbf{r}, t) \exp(i\omega_0 t - t/T_2) \quad [2.36]$$

It follows that Eqn. 2.35 reduces to

$$\frac{\partial m_+}{\partial t} = -i \gamma (\mathbf{G} \cdot \mathbf{r}) m_+ + D \nabla^2 m_+ \quad [2.37]$$

In the absence of diffusion and with the boundary condition that $m_+ = A$ after the first rf pulse, the solution of Eqn. 2.37 is

$$m_+ = A \exp \left(-i \gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt' \right) \quad [2.38]$$

The exponential term in Eqn. 2.38 refers to the accumulated phase shift of a spin at position $\mathbf{r}$ that experienced a gradient ($\mathbf{G}$) over a time t . Diffusion makes A a function of time. Substitution of Eqn. 2.38 into Eqn. 2.37 yields

$$\frac{\partial A(t)}{\partial t} = -D \gamma^2 \left(\int_0^t \mathbf{G}(t') dt' \right)^2 A(t) \quad [2.39]$$

which has the following solution

$$A(t) = A_0(t) \exp \left[-D \gamma^2 \int_0^t \left(\int_0^{t'} \mathbf{G}(t'') dt'' \right)^2 dt' \right] \quad [2.40]$$

where $A_0(t)$ is the echo amplitude in the absence of gradients. In addition, $A_0(t)$ equals $A_0 \exp(-t/T_2)$ where A_0 is the transverse magnetization immediately after the first 90° rf pulse. In the general case of free diffusion where the diffusion cannot be characterized by a single diffusion coefficient (D), but, instead is characterized by a diffusion tensor ($\mathbf{D}$), Eqn. 2.40 becomes

$$A(t) = A_0(t) \exp \left(-\gamma^2 \int_0^t \left\{ \left[\int_0^{t'} \mathbf{G}(t'') dt'' \right] \cdot \mathbf{D} \cdot \left[\int_0^{t'} \mathbf{G}(t'') dt'' \right] \right\} dt' \right) \quad [2.41]$$

In order to treat the role of 180° refocussing rf pulses, the rf pulse is ignored and, instead, the sign of the effective gradient, $\mathbf{G}(t')$, is inverted following a 180° rf pulse. Eqns. 2.40 and 2.41 relate the spin-echo amplitude $\{A(t)\}$ to the strength and duration of the magnetic field gradients, and to the diffusion coefficient (or tensor).

Consider the response of the transverse magnetization of isotropic, freely diffusing spins to a spin-echo pulse sequence with a constant gradient, $\mathbf{G}_0$. We need to calculate the argument of the exponential in Eqn. 2.40.

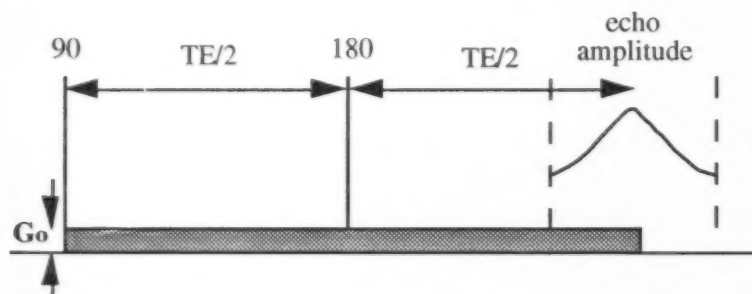


Figure 2.5 Spin-echo pulse sequence with a constant gradient, $\mathbf{G}_0$. TE refers to the time between the 90° rf pulse and the echo maximum.

Evaluating $\int_0^{t'} G(t'') dt''$ (i.e. the area of the gradient) yields

$$\begin{aligned} G_0 t' & \quad 0 < t' < TE/2 \\ G_0(TE/2) - G_0(t' - TE/2) = G_0(TE - t') & \quad TE/2 < t' < TE \end{aligned}$$

$$\text{and } \left(\int_0^{t'} G(t'') dt'' \right)^2 \text{ yields}$$

$$\begin{aligned} G_0^2 t'^2 & \quad 0 < t' < TE/2 \\ G_0^2(TE^2 - 2TEt' + t'^2) & \quad TE/2 < t' < TE \end{aligned}$$

Now evaluating $\int_0^{TE} \left(\int_0^{t'} G(t'') dt'' \right)^2 dt'$ over the limits of integration presented above

and substituting the result into Eqn. 2.40 yields

$$A(t) = A_0(t) \exp \left\{ -\frac{2}{3} \gamma^2 G_0^2 (TE/2)^3 D \right\} \quad [2.42]$$

To take relaxation into account, $A_0(t)$ is replaced by $A_0 \exp(-t/T_2)$ and Eqn. 2.42 yields an echo amplitude $\{A(TE)\}$ of

$$A(TE) = A_0 \exp \left\{ -TE/T_2 - \frac{2}{3} \gamma^2 G_0^2 (TE/2)^3 D \right\} \quad [2.43]$$

Assuming that T_2 and the magnitude of G_0 are known, D can be calculated by monitoring $A(TE)$ as a function of the echo time TE . Larger magnetic field gradients (G_0) are required to measure smaller D values.

The measurement of D via the constant gradient technique has several disadvantages. First, the precise period of time during which diffusion is measured is ambiguous because the spin-echo time is incremented throughout the measurement.

There are problems with isolating the attenuation due to T_2 from that due to diffusion. The amplitude of the spin-echo can be difficult to measure accurately since the echo will be very sharp in a strong static inhomogeneous field. The acquisition of sharp echoes requires broadband circuitry which is inherently noisy. Also, one cannot excite uniformly all of the spins in a sample experiencing a large magnetic field gradient. The aforementioned limitations restrict the lower limit of D values attainable by the constant gradient technique. However, the *pulsed-gradient spin-echo* technique for measuring diffusion overcomes many of the above limitations (Stejskal 1965).

Note that Carr and Purcell calculated the echo attenuation for a series of n consecutive 180° rf pulses at times $TE/2$, $3(TE/2)$, $5(TE/2)$, etc. with subsequent echoes at TE , $2TE$, $3TE$, etc. (Carr 1954). The echo amplitude of the n th echo occurring at a time nTE is

$$A(nTE) = A_0 \exp \left\{ -\frac{nTE}{T_2} - \frac{\gamma^2 G_0^2 TE^3 D}{12n^2} \right\} \quad [2.44]$$

Therefore, if the focus is measuring T_2 , then the effects of diffusion on the echo amplitudes can be minimized by applying a large number (n) of closely spaced (i.e. small TE) 180° rf pulses.

2.1.5.3 Pulsed-Gradient Spin-Echo Method to Measure Diffusion

The pulsed-gradient spin-echo (PGSE) diffusion pulse sequence (Fig. 2.6) was proposed by Stejskal and Tanner (Stejskal 1965). This simple pulse sequence forms the basis of most of the diffusion measurements that are presented in this thesis. PGSE differs from the constant gradient method in that the gradients used to measure diffusion are not applied during the application of the rf pulses or during the acquisition of the echo. The echo attenuation, as a function of the timings and strengths of the rectangular gradient pulses and the local gradients, and the diffusion coefficient, will be evaluated for the PGSE pulse sequence (Fig. 2.6) using the formalism derived in Section 2.1.5.2. The local gradients, G_0 , (often referred to as background gradients in the literature) are due to static inhomogeneities in the main magnetic field and due to local magnetic-susceptibility-differences within the sample.

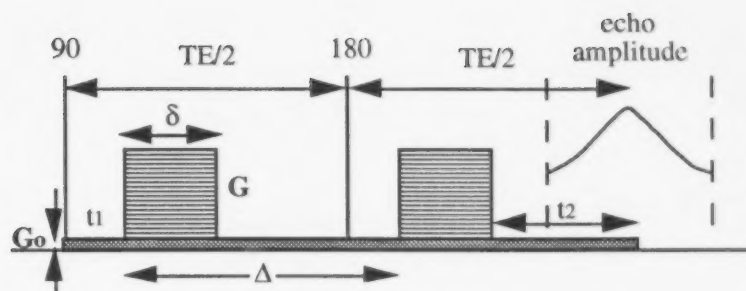


Figure 2.6 The basic pulsed-gradient spin-echo (PGSE) pulse sequence for measuring diffusion (Stejskal 1965). δ is the length of the applied rectangular diffusion-sensitizing gradient pulses, Δ the separation between the onsets of the two gradient pulses, and G is the gradient strength of the applied gradient. The gradient pulses need not be rectangular to measure diffusion. G_0 is the strength of the local gradients.

Evaluating $\int_0^{t'} G(t'') dt''$ (i.e. the area of the gradient) for the PGSE sequence illustrated

in Fig. 2.6 and remembering that the signs of the gradients are reversed after the 180° rf pulse yields,

$G_0 t'$	$0 < t < t_1$
$G_0 t' + G(t' - t_1)$	$t_1 < t < t_1 + \delta$
$G_0 t' + G\delta$	$t_1 + \delta < t < TE/2$
$G_0(TE/2) - G_0(t' - TE/2) + G\delta$	$TE/2 < t < t_1 + \Delta$
$\quad = G_0(TE - t') + G\delta$	
$G_0(TE/2) - G_0(t' - TE/2) + G\delta - G\{t' - (t_1 + \Delta)\}$	$t_1 + \Delta < t < t_1 + \Delta + \delta$
$\quad = G_0(TE - t') + G(\delta - t' + t_1 + \Delta)$	
$G_0(TE/2) - G_0(t' - TE/2) + G\delta - G\delta$	$t_1 + \Delta + \delta < t < TE$
$\quad = G_0(TE - t')$	

$$\text{and } \left(\int_0^{t'} G(t'') dt'' \right)^2 \text{ yields}$$

$$\begin{array}{ll} G_o^2 t'^2 & 0 < t < t_1 \\ G_o^2 t'^2 + 2G_o G t'(t' - t_1) + G^2(t'^2 - 2t't_1 + t_1^2) & t_1 < t < t_1 + \delta \\ G_o^2 t'^2 + 2G_o G t'\delta + G^2 \delta^2 & t_1 + \delta < t < TE/2 \\ G_o^2(TE - t')^2 + 2G_o G(TE - t')\delta + G^2 \delta^2 & TE/2 < t < t_1 + \Delta \\ G_o^2(TE - t')^2 + 2G_o G(TE - t')(\delta - t' + t_1 + \Delta) & \\ + G^2(\delta - t' + t_1 + \Delta)^2 & t_1 + \Delta < t < t_1 + \Delta + \delta \\ G_o^2(TE - t')^2 & t_1 + \Delta + \delta < t < TE \end{array}$$

$$\text{Now, } \int_0^{TE} \left(\int_0^{t'} G(t'') dt'' \right)^2 dt' \text{ evaluated over the limits of integration presented above}$$

yields (in order of the above six ranges of time),

$$\begin{array}{l} G_o^2 t_1^3/3 \\ G_o^2(t_1^2\delta + \delta^2 t_1 + \delta^3/3) + G_o G(2\delta^3/3 + \delta^2 t_1) + G^2 \delta^3/3 \\ G_o^2/3 \{ (TE/2)^3 - (t_1 + \delta)^3 \} + G_o G(\delta TE^2/4 - \delta t_1^2 - 2t_1 \delta^2 - \delta^3) + G^2(\delta^2 TE/2 - \delta^2 t_1 - \delta^3) \\ G_o^2 \{ TE^2 t_1 + TE^2 \Delta - TE t_1^2 - 2TE t_1 \Delta - TE \Delta^2 + (t_1 + \Delta)^3/3 - 7TE^3/24 \} + \\ G_o G(2TE t_1 \delta + 2TE \Delta \delta - t_1^2 \delta - 2t_1 \Delta \delta - \Delta^2 \delta - 3TE^2 \delta/4) + G^2(\delta^2 t_1 + \delta^2 \Delta - \delta^2 TE/2) \\ G_o^2 \{ TE^2(t_1 + \Delta + \delta) - TE(t_1 + \Delta + \delta)^2 + (t_1 + \Delta + \delta)^3/3 - TE^2(t_1 + \Delta) + TE(t_1 + \Delta)^2 - (t_1 + \Delta)^3/3 \} + G_o G(TE \delta^2 - \delta^3/3 - \delta^2 t_1 - \delta^2 \Delta) + G^2 \delta^3/3 \\ G_o^2 \{ TE^3/3 - TE^2(t_1 + \Delta + \delta) + TE(t_1 + \Delta + \delta)^2 - (t_1 + \Delta + \delta)^3/3 \} \end{array}$$

Summing these six terms, collecting terms with respect to G_o^2 , $G_o G$, and G^2 , substituting into Eqn. 2.40, and taking the natural logarithm of both sides of the equation yields

$$\ln \left\{ \frac{A(TE, G)}{A(TE, G=0)} \right\} = -\gamma^2 D \left\{ \frac{2}{3}(TE/2)^3 G_0^2 - \delta \left[\frac{2}{3}\delta^2 - 2(TE/2)^2 + (t_1^2 + t_2^2) + \delta(t_1 + t_2) \right] G_0 \cdot G + \delta^2(\Delta - \delta/3)G^2 \right\} \quad [2.45]$$

$A(TE, G)$ and $A(TE, G=0)$ are the echo amplitudes with and without the application of a magnetic field gradient, respectively. Of course, the echo amplitudes at TE are still attenuated by $\exp(-TE/T_2)$. However, the influence of T_2 on the measured diffusion coefficient is removed by taking the ratio of the aforementioned amplitudes.

If we ignore the applied gradients, G , Eqn. 2.45 reduces to that obtained for the constant gradient experiment (Eqn. 2.42), as expected. In the absence of local gradients, G_0 , Eqn. 2.45 reduces to

$$\ln \left\{ \frac{A(TE, G)}{A(TE, G=0)} \right\} = -\gamma^2 \delta^2 G^2 (\Delta - \delta/3) D = -b D \quad , \quad [2.46]$$

where the portion $\gamma^2 \delta^2 G^2 (\Delta - \delta/3)$ is usually called the gradient factor, b (Le Bihan 1986). Eqn. 2.46 forms the basis of the great majority of the diffusion measurements which are presented in this thesis. The local gradients are assumed to be minimal in our samples. This is true for a homogeneous sample, such as pure water, but may not necessarily be true for inhomogeneous tissue samples which may have susceptibility-difference-induced magnetic field gradients. The evaluation of the contribution of local gradients on the measured D values in our nerve samples is presented in Section 4.3.

The effective diffusion time, t_d , is given by $\Delta - \delta/3$ and it is often in the range of 1-100 ms which corresponds to root-mean-square displacements of 2 - 20 μm for pure water. In order to measure D , the timings δ or Δ can be varied, but generally the timings are kept constant and the gradient strength is varied to measure diffusion. Since only the diffusion parallel to a gradient can cause signal attenuation, the diffusion coefficients along different directions in the sample can be measured by applying the pulsed gradients along distinct spatial axes. The echo time, TE, is kept constant in order to keep the same degree of echo attenuation due to relaxation. For isotropic, unrestricted diffusion, a plot of $\ln \left\{ \frac{A(TE, G)}{A(TE, G=0)} \right\}$ versus b should yield a straight line with slope $-D$. However, typical NMR measurements of diffusion in biological tissues do not yield the "true" intrinsic diffusion coefficient of water in the

tissue because of restrictions by cellular structures. Nonetheless, Eqn. 2.46 is still useful and the measured diffusion coefficient in tissue is usually denoted by the apparent diffusion coefficient (ADC) or, as we prefer to call it in this thesis, D_{NMR} . In some instances, the decay of the signal amplitude can deviate from being a monoexponential function of b . Curvature in the semilogarithmic diffusion decay curves could be due to multiple independent (or slowly exchanging) compartments with differing diffusive abilities, significant local gradients (G_0), and/or restricted diffusion (Karger 1988b, Zhong 1991a, Le Bihan 1991, Hürlimann 1995).

In general, diffusion in 3-dimensions is described by a diffusion tensor of rank two ($\mathbf{D}$) (Basser 1994a, Basser 1994b),

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} . \quad [2.47]$$

One can measure the diagonal elements D_{xx} , D_{yy} , or D_{zz} by applying the pulsed gradients in the x , y , or z direction, respectively. An isotropic medium would yield $D_{xx} = D_{yy} = D_{zz}$. The off-diagonal elements require the combined application of gradients in two different directions. For an isotropic medium, the off-diagonal elements are zero. However, this is not necessarily true for an anisotropic medium. The off-diagonal elements will be zero in an anisotropic medium only if the principal axes of the medium are aligned with the coordinates axes of the laboratory frame. Therefore, assuming we have a cylindrical sample whose long axis is parallel ($//$) to the x -axis of the lab frame, the diffusion tensor simplifies to

$$\mathbf{D} = \begin{bmatrix} D_{//} & 0 & 0 \\ 0 & D_{\perp} & 0 \\ 0 & 0 & D_{\perp} \end{bmatrix} . \quad [2.48]$$

Therefore, there are only two independent elements describing diffusion in this case (i.e. diffusion parallel to the axis of the cylinder ($D_{//}$) and diffusion perpendicular to it ($D_{\perp}$)). An anisotropic diffusion ratio, $\frac{D_{//}}{D_{\perp}}$, can be defined to give a measure of the degree of anisotropy in the system. Of course, an anisotropic diffusion ratio of 1 reflects isotropic diffusion.

In order to neglect orientational effects, an *average* diffusion coefficient (D_{av}) can be calculated by measuring diffusion in three orthogonal directions and then adding the three resulting diagonal elements to calculate the trace of the diffusion tensor (van Gelderen 1994a). For a cylinder,

$$D_{av} = 1/3 \text{Tr}(\mathbf{D}) = 1/3 (D_{//} + 2D_{\perp}) \quad [2.49]$$

where $\text{Tr}(\mathbf{D})$ represents the trace of the diffusion tensor. In fact, with the appropriate pattern of gradients, the trace of the diffusion tensor can be obtained in a single acquisition (Mori 1995).

The PGSE measurement of diffusion can be visualized as follows. The two gradient pulses, if short enough with respect to the distance between them (i.e. $\delta \ll \Delta$), can be treated as delta functions. The first applied gradient can be thought of as an instantaneous pulse which *labels* each nucleus by a phase shift. The phase depends on the position of the nucleus in the field gradient at the time of the pulse. If the local gradients are negligible, the loss of phase coherence after the gradient pulse is insubstantial. The 180° rf pulse inverts the phase shifts. In the absence of diffusion, the second gradient pulse, if it is of equal area to the first gradient pulse, completely refocusses the phase shifts. As illustrated in Fig. 2.4, diffusion causes the refocussing to be incomplete. Only the changes in position during the time between the two gradient pulses are important for incomplete refocussing and the attenuation of the echo amplitude.

There are several advantages of the PGSE experiment over its constant gradient counterpart. (a) The decay due to relaxation can be kept constant which greatly simplifies the measurement of D . (b) The diffusion time (t_d) is defined more precisely. Subsequently, t_d can be incremented in order to test for restrictive influences of internal barriers on diffusion. (c) The applied gradients can be made larger since they are not on during the rf pulses or the echo acquisition. This permits the measurement of smaller diffusion coefficients. Also, since the magnetization only experiences a small G_0 (i.e. homogeneous field) during acquisition of the echo, the echo is broader and hence, its amplitude is easier to measure accurately. The advantages of the PGSE method of measuring diffusion, as compared to traditional isotope-labeled methods,

are the lack of perturbation of the system and the rapidity of the measurement procedure.

The measurement of diffusion by the PGSE method requires very stable and precise gradient pulses. Additional echo attenuation is observed if the first and second gradient pulses are mismatched, resulting in diffusion coefficients that are larger than expected. Often, the second gradient pulse needs to be adjusted so that the pulse sequence yields an echo with maximum amplitude centered at the appropriate echo time. In the literature, there is an excellent example of the marked effects on the echo shape and amplitude that can occur when the first and second gradient pulses are mismatched marginally (Fig. 4 in Cooper 1974). Due to the maximum attainable voltage and bandwidth of the rf amplifier, and the ratio of inductance over resistance of the gradient coils, gradient pulses have finite rise and fall times, and therefore, are not perfectly rectangular (Damji 1995). The effect of non-rectangular gradient pulses on the measured diffusion coefficient has been shown to be negligible (Price 1991). The important point is that the *area* of the non-rectangular pulse should be equal to that of the ideal rectangular pulse (i.e. $G\delta$). Eddy currents can also induce additional signal attenuation. Therefore, it is important to minimize any vibrations of wires comprising the rf coil or the gradient coils in order to avoid troublesome eddy currents. Some of the experimental pitfalls of the PGSE method and proposed solutions are discussed in a series of papers by Hrovat and Wade (Hrovat 1981a, Hrovat 1981b). *Therefore, good hardware for producing gradients is essential!*

The strength of the gradient pulses must be accurately known. One of the best and easiest ways to calibrate the gradients, independent of diffusion, is the "FID zero" method of Murday (Murday 1973). The "FID zero" method involves observing the free induction decay (FID) or a spin-echo in the presence of a field gradient using a cylindrical sample (of radius R) that is orthogonal to the gradient. The FID waveform is described by a first-order Bessel function. By utilizing the zeroes (α_i) of a first-order Bessel function, the gradient strength (G) can be calibrated from

$$G = \frac{\alpha_i}{\gamma R t_i} \quad [2.50]$$

or from the spacing between consecutive zeroes of the FID

$$G = \frac{\alpha_{i+1} - \alpha_i}{\gamma R (t_{i+1} - t_i)} \quad [2.51]$$

where t_i is the time of the i th zero-crossing of the FID. Another commonly used method of gradient calibration involves using the PGSE sequence to measure the echo attenuations of a sample with a known diffusion coefficient, and working backwards to calculate G (using Eqn. 2.46).

As mentioned earlier, local gradients (G_o) are usually ignored and Eqn. 2.46 is used to calculate the diffusion coefficient. However, if G_o is substantial and is not negligible with respect to G , Eqn. 2.45 must be used since it contains the contributions of local gradients to the echo attenuation. The magnitude and direction of the local gradients are not known, in general. It is preferable in some situations, therefore, to use a pulse sequence that eliminates or reduces the effect of local gradients on the measured D . Variants of the PGSE pulse sequence have been proposed for this purpose (Williams 1978, Karlicek 1980, Hong 1992). The bipolar gradient pulse (BGP) sequence, as proposed by Hong and Dixon (Hong 1992), is the simplest of these methods. BGP will be discussed in Section 4.3 when we use it to test the contribution of local gradients to the measured diffusion coefficients in our nerve samples.

We have only discussed diffusion methodologies that measure the amplitude of the spin-echo. In addition, it is possible to measure diffusion via the stimulated echo (Tanner 1970). Stimulated echo methods have been introduced to eliminate the effects of local gradients on diffusion measurements (Cotts 1989). The stimulated echo diffusion technique has one distinct advantage over the spin-echo diffusion technique. The relaxation dependence of the stimulated echo is heavily T_1 weighted, as opposed to T_2 weighted. Therefore, this may be of some advantage in systems where $T_1 > T_2$ because there will be less signal loss due to relaxation. The stimulated echo sequence may also allow longer diffusion times and measurements of smaller D values. The loss of 50% of the original equilibrium magnetization, due to dephasing of the spins during the pulse sequence, is one major disadvantage of the stimulated echo. The stimulated echo sequence was not used for the measurements of diffusion presented in

this thesis because adequate signal-to-noise was obtained for our nerve samples using the simpler spin-echo technique.

The introduction of diffusion-weighted imaging in the mid 1980's provided a novel contrast mechanism for MRI, as well as a non-invasive method of measuring the mobility of water in various tissues, *in-vivo* (Wesbey 1984, Merboldt 1985, Taylor 1985, Le Bihan 1986). The basics of diffusion-weighted MR imaging (DWI) and some potential clinical applications are presented in several review papers (Le Bihan 1991, Le Bihan 1992, Moseley 1991a, Moseley 1992). The clinical applications and uses of DWI are presented in Section 2.2.2. Diffusion-sensitizing gradient pulses are simply added to typical imaging sequences like the two-dimensional Fourier transform spin-echo method. However, the application of DWI *in-vivo* presents two primary difficulties. First, clinical MR scanners are usually equipped with weak gradients, and therefore, the available gradient strengths may be insufficient to obtain an adequate degree of echo attenuation due to diffusion. The lengths of the gradient pulses and their spacing are usually made longer in order to compensate for weak gradient strengths. However, this increases the echo time and hence, the signal-to-noise will decrease accordingly. Also, the assumption that $\delta \ll \Delta$ no longer holds. Smaller-diameter gradient coils that can be inserted into the main bore of the magnet are often used to get increased gradient strengths. Also, the use of recent technological improvements such as shielded gradient coils reduces eddy currents and should improve the accuracy of diffusion measurements. Second, since we are measuring diffusion distances of the order of tens of μm , it is important to avoid any motion of the patient or sample. Extremely fast imaging techniques, such as echo-planar imaging (EPI) (Mansfield 1977, Turner 1990a, Turner 1990b), can be used to minimize the deleterious effects of sample motion during acquisition of the image. If routine MR imaging methods are utilized, there are techniques, such as the navigator echo, available for correcting image artifacts due to motion (Ordidge 1994, Anderson 1994). In summary, diffusion-weighted MRI is feasible and has been used extensively. A survey of diffusion measurements in tissue (particularly nervous tissue), both *in-vitro* and *in-vivo*, will be provided in Section 2.2.2.

2.1.5.4 q-Space Imaging

The PGSE pulse sequence essentially measures the net root-mean-square displacement of the particles. Usually, the PGSE data obtained by incrementing the gradient strength is analyzed by plotting $\ln(\text{echo amplitude})$ versus the gradient factor, b . The slope of this plot yields the diffusion coefficient (D). Alternatively, the PGSE data can be processed to yield the actual displacement distributions instead of simply a diffusion coefficient (Callaghan 1988, Callaghan 1991b, Cory 1990). This data analysis is called **q-space "imaging"** and it yields information about coherent (i.e. flow) and incoherent (i.e. diffusion) water displacements. We will neglect the consideration of flow in the following discussion since it is not directly pertinent to this thesis.

Consider the case of an initial density of spins at various positions, $\rho(\mathbf{r})$. A conditional probability, $P_s(\mathbf{r} | \mathbf{r}', \Delta)$, describes the likelihood that a spin initially at position $\mathbf{r}$ will move to position $\mathbf{r}'$ in a time Δ . If the gradient pulses in the PGSE sequence are considered instantaneous, then Δ is the time between them and represents the diffusion time. The mobile spins that move from $\mathbf{r}$ to $\mathbf{r}'$ experience a net phase shift of $\gamma\delta\mathbf{G} \cdot (\mathbf{r}' - \mathbf{r})$. Hence, the echo amplitude, $E_\Delta(\mathbf{G})$ (using Callaghan's symbols), is given by

$$E_\Delta(\mathbf{G}) = \int \rho(\mathbf{r}) \int P_s(\mathbf{r} | \mathbf{r}', \Delta) \exp\{i \gamma\delta\mathbf{G} \cdot (\mathbf{r}' - \mathbf{r})\} d\mathbf{r}' d\mathbf{r} \quad . \quad [2.52]$$

An "averaged propagator" $\{\bar{P}_s(\mathbf{r} | \mathbf{r}', \Delta)\}$, as it is called, can be defined as the spatial average of $P_s(\mathbf{r} | \mathbf{r}', \Delta)$ and by replacing $\mathbf{r}' - \mathbf{r}$ with $\mathbf{R}$, Eqn. 2.52 reduces to

$$E_\Delta(\mathbf{G}) = \int \bar{P}_s(\mathbf{R}, \Delta) \exp(i \gamma\delta\mathbf{G} \cdot \mathbf{R}) d\mathbf{R} \quad . \quad [2.53]$$

Defining $\mathbf{q} = (1/2\pi)\gamma\delta\mathbf{G}$, Eqn. 2.53 becomes

$$E_\Delta(\mathbf{q}) = \int \bar{P}_s(\mathbf{R}, \Delta) \exp(i 2\pi \mathbf{q} \cdot \mathbf{R}) d\mathbf{R} \quad . \quad [2.54]$$

Equation 2.54 describes a Fourier relationship between $E_{\Delta}(\mathbf{q})$ and $\bar{P}_s(\mathbf{R}, \Delta)$. The term $\mathbf{q}$ -space imaging comes from the analogy with $\mathbf{k}$ space of normal MR imaging in which the signal, $S(\mathbf{k})$, is sampled in $\mathbf{k}$ -space and is related by a Fourier transform to the image density, $\rho(\mathbf{r})$. The PGSE experiment can, therefore, be thought of as an "imaging" experiment in which data sampling occurs in $\mathbf{q}$ -space. After Fourier transformation, the resulting "image" of $\bar{P}_s(\mathbf{R}, \Delta)$ represents the displacement probabilities of the particles.

In the case of unrestricted Brownian motion of a single component, the solution of Eqn. 2.54 is

$$E_{\Delta}(\mathbf{q}) = \exp(-4\pi^2 \mathbf{q}^2 \Delta D) \quad [2.55]$$

which is equivalent to the narrow-pulse approximation of the previously derived result in Eqn. 2.46 (i.e. use Δ in this case instead of $\Delta\delta/3$). The $\mathbf{q}$ -space Fourier transform of Eqn. 2.55 yields (Callaghan 1991b)

$$\bar{P}_s(\mathbf{R}, \Delta) = (4\pi D \Delta)^{-1/2} \exp(-\mathbf{R}^2 / 4D\Delta) \quad [2.56]$$

which is equivalent to the particle distribution derived from Fick's second law of diffusion (see Eqn. 2.29). Hence, the calculated displacement profile, in this case, is a Gaussian peak. The width of the Gaussian is proportional to the square-root of the diffusion coefficient. Subsequently, D can be calculated from the full-width at half-maximum (FWHM) of the Gaussian peak (Cory 1990)

$$D = \frac{\text{FWHM}^2}{16 \Delta \ln(2)} \quad [2.57]$$

It is interesting to note that in the presence of flow, the shape of the peak in the displacement profile is unaltered, but its position is shifted in proportion to the velocity of the flow.

For $\mathbf{q}$ -space imaging, the important experimental features for data acquisition are as follows. (a) The echo amplitudes must be acquired with evenly spaced gradient increments or the data must be interpolated post-acquisition to get equally spaced $\mathbf{q}$

values. This even-spacing is a requirement for the Fourier transform. (b) In order for the Fourier relationship to hold, the gradient pulses should be as short as possible. (c) Gradients should be strong enough to fully attenuate the echo amplitude in order to avoid truncation artifacts after the Fourier transform.

Two different approaches of analyzing the PGSE data in order to measure the diffusion coefficient and to get an idea of the displacement profile of diffusing particles are illustrated in Fig. 2.7.

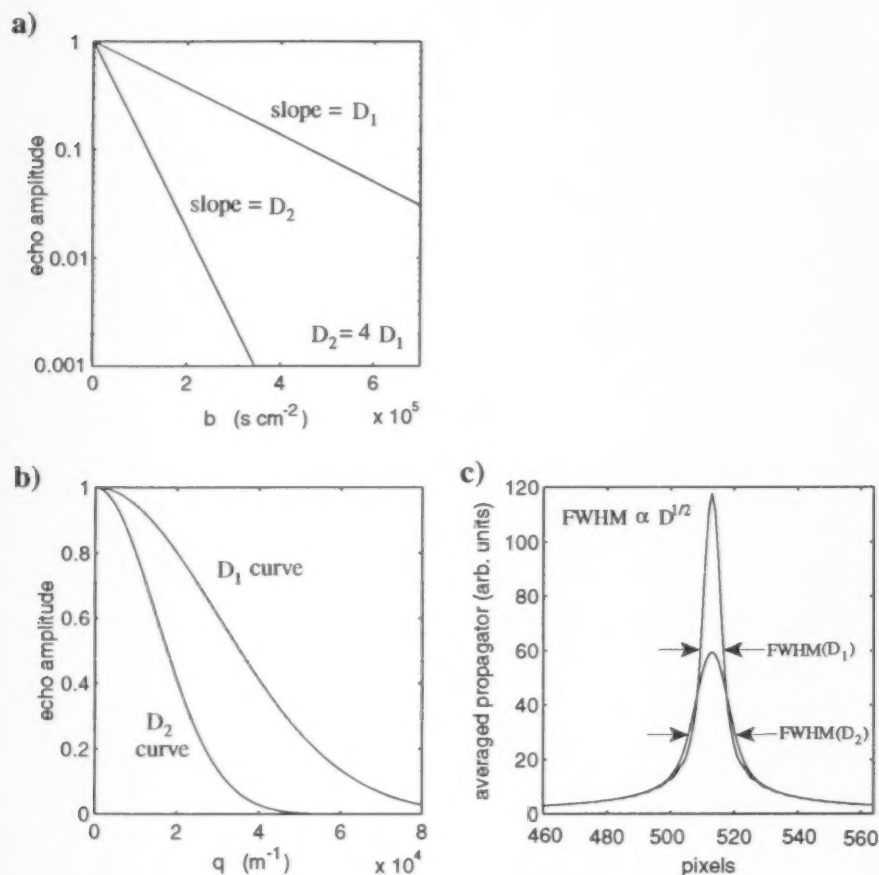


Figure 2.7 A comparison of two methods of processing PGSE data for two samples with diffusion coefficients that differ by a factor of four (i.e. $D_2 = 4 \cdot D_1$). (a) The routine method of measuring the diffusion coefficient from the slope of a $\ln(\text{echo amplitude})$ vs. b -value plot. The faster diffusing substance (D_2) has a greater slope (i.e. loses signal more quickly). (b) Alternatively, the echo amplitudes are plotted against q instead of b . (c) The discrete Fourier transform (1024 points in this case) of the curves in (b) yields Gaussian distributions for the averaged propagator (i.e. displacement probability), as expected for Brownian motion. The full-width at half-maximum (FWHM) of the Gaussian is proportional to the square-root of the diffusion coefficient. Hence, the FWHM for D_2 is twice the FWHM for D_1 . The faster diffusing particles have a greater probability of moving further from the origin (i.e. pixel 512, the center of the 1024 point Fourier transform).

2.1.6 Magnetization Transfer

The nuclear Overhauser effect (nOe) involves the change in intensity of the signal (i.e. net magnetization) of one resonant species when another nuclear species is irradiated with a radiofrequency field (rf). This effect is due to population changes of the nuclear energy levels, and hence, involves spin-lattice relaxation mechanisms which attempt to re-establish thermal equilibrium after the initial perturbation of the spin system. The two nuclear species communicate with one another via cross-relaxation or magnetization transfer (MT), as it is commonly called in the in-vivo NMR community. Therefore, the spin-lattice relaxation portion of Section 2.1.4 must be expanded to account for magnetization transfer.

Consider two unique spin-1/2 nuclei, I and S, with the same γ and assume that they are not scalar coupled. We assume that the dipole-dipole interaction is the main mode of relaxation. The energy levels of the IS spin system and the transition probabilities per unit time (W) between the energy levels are illustrated in Fig. 2.8. W_1^I and W_1^S are the probabilities of single quantum transitions that invert spins I or S, respectively, and leave the other spin unchanged. W_0 is the flip-flop term and represents the probability of simultaneous transitions of both spins in opposite directions. W_2 is the probability of simultaneous transitions of both spins in the same direction. The numerical subscripts of the W's represents the change in total spin of the system during a transition.

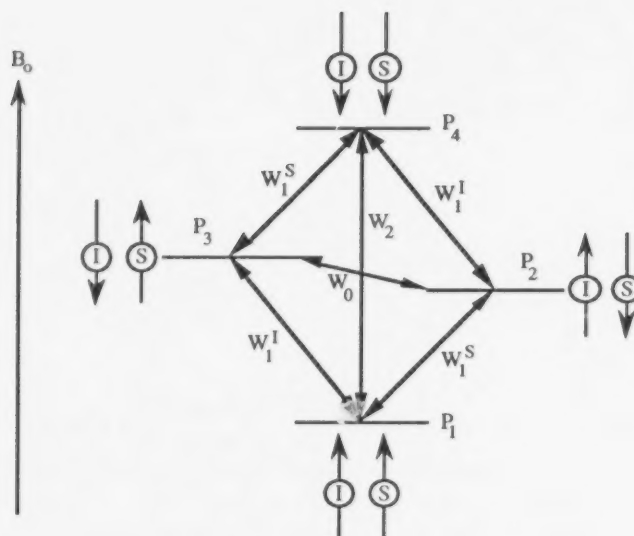


Figure 2.8 The energy levels in a spin system consisting of two uncoupled spin-1/2 nuclei, I and S, in a field B_0 . The probabilities per unit time (W) of the six possible transitions between the four energy levels are labeled. The populations of the levels are given by P_1 , P_2 , P_3 , and P_4 . At equilibrium, $P_1 > P_2 \sim P_3 > P_4$. The population differences $P_1 - P_2$ and $P_3 - P_4$ become zero after saturation of the S nuclei with rf. Subsequently, the spin-lattice relaxation pathways redistribute the populations of the levels in order to re-establish thermal equilibrium. If the cross-relaxation pathways, W_2 and W_0 , are the active and dominant relaxation pathways, the population differences for the transitions of I (i.e. $P_1 - P_3$ and $P_2 - P_4$) can change, and hence, can alter the intensity of the signal from I.

If I and S relax **independently**, the rate of change of the ensemble-averaged longitudinal magnetization (i.e. along the z-axis) of I and S is given by

$$\frac{d\langle I_z \rangle}{dt} = -\rho_I (\langle I_z \rangle - I_0) \quad [2.58a]$$

$$\frac{d\langle S_z \rangle}{dt} = -\rho_S (\langle S_z \rangle - S_0) \quad [2.58b]$$

where ρ_I and ρ_S are known as the self-relaxation rates of I and S, respectively. I_0 and S_0 are the values of the equilibrium magnetization. Eqns. 2.58a and 2.58b are very similar in form to Eqn. 2.21 where $\rho = R_1 (= 1/T_1)$.

In general, if the relaxation rate of one type of nucleus, I, depends on the population differences of the other nucleus, S, and vice-versa, an additional term must be added to Eqn. 2.58. Therefore,

$$\frac{d\langle I_z \rangle}{dt} = -\rho_I (\langle I_z \rangle - I_0) - \sigma_{IS} (\langle S_z \rangle - S_0) \quad [2.59a]$$

$$\frac{d\langle S_z \rangle}{dt} = -\rho_S (\langle S_z \rangle - S_0) - \sigma_{IS} (\langle I_z \rangle - I_0) \quad [2.59b]$$

where σ_{IS} is called the **cross-relaxation** rate. It can be shown that the self- and cross-relaxation rates are related to the transition probabilities (Noggle 1971, Bovey 1988),

$$\rho_I = W_0 + 2W_1^I + W_2 \quad [2.60a]$$

$$\rho_S = W_0 + 2W_1^S + W_2 \quad [2.60b]$$

$$\sigma_{IS} = W_2 - W_0 \quad [2.60c]$$

If only single quantum transitions were active as relaxation pathways (i.e. $\sigma_{IS} = 0$), saturating S with rf would not affect the intensity of I (i.e. there would be no nOe). Therefore, cross-relaxation or magnetization transfer is necessary for the nuclear Overhauser effect.

Consider the case when S is saturated selectively with rf (i.e. $\langle S_z \rangle = 0$), and the intensity of I is monitored at steady-state (i.e. $d\langle I_z \rangle/dt = 0$). Utilizing these starting conditions and substituting into Eqn. 2.59a, the nOe enhancement $\{\eta_I(S)\}$ of the signal for spin I when S is saturated ($\langle I_z \rangle$), with respect to the equilibrium intensity of spin I (I_0), is given by (Solomon 1955, Noggle 1971)

$$\eta_I(S) = \frac{\langle I_z \rangle - I_0}{I_0} = \frac{\sigma_{IS} S_0}{\rho_I I_0} \quad [2.61a]$$

where $I_0 \propto I(I+1)\gamma_I$ and $S_0 \propto S(S+1)\gamma_S$ {I and S are the spin quantum numbers and γ_I and γ_S are the magnetogyric ratios for spins I and S}. Now, $I = S = 1/2$ and $\gamma_I = \gamma_S$

for two interacting protons. Therefore, S_0/I_0 is unity and Eqn. 2.61a becomes (Noggle 1971)

$$\eta_I(S) = \frac{\sigma_{IS}}{\rho_I} = \frac{W_2 - W_0}{W_0 + 2W_1^I + W_2} \quad [2.61b]$$

The transition probabilities are a function of the correlation time (τ_c) (defined in Section 2.1.4) of the molecules of interest (Bovey 1988). W_0 is the dominant relaxation pathway for macromolecules (large τ_c), and hence, $\eta_I(S)$ is negative and saturating S decreases the signal of I (i.e. negative nOe). Therefore, a negative nOe is expected for water that is interacting with macromolecules in tissue. In contrast, W_2 is the dominant relaxation pathway for small molecules (small τ_c), and hence, $\eta_I(S)$ is positive and saturating S increases the signal of I (i.e. positive nOe). Interestingly, zero nOe's can be obtained for intermediate cases where $W_2 \sim W_0$ (i.e. $\sigma_{IS} \sim 0$). There is a nice intuitive description of the nOe in the book by Derome (Derome 1987). Therefore, the importance of cross-relaxation to the overall relaxation of a nuclear spin system can be evaluated using selective rf saturation.

Magnetization transfer (MT) plays an integral role in the relaxation mechanism of water protons in tissue (Edzes 1977, Edzes 1978). In particular, consider a two-pool model consisting of a free water pool (H_f) and a restricted semi-solid macromolecular pool (H_r) (Fig. 2.9). H_f and H_r are coupled via chemical exchange and/or dipole-dipole interactions, and hence, can exchange magnetization. The macromolecular protons are saturated selectively with rf and the intensity of the magnetization of the free water protons is monitored. If the intensity of the free water protons decreases significantly from its equilibrium value (i.e. negative nOe), then magnetization transfer between H_r and H_f must be an important factor for relaxation in the tissue of interest. The study of magnetization transfer will hopefully improve the understanding of relaxation in various tissues, normal and abnormal. In addition, MT provides an alternative contrast mechanism in MRI, as first demonstrated by Wolff and Balaban (Wolff 1989). A review of magnetization transfer contrast in MRI and of some of the basic concepts of MT is available in the literature (Balaban 1992). Some of the salient points of MT, as they pertain to this thesis, will be discussed in the following portions of this section.

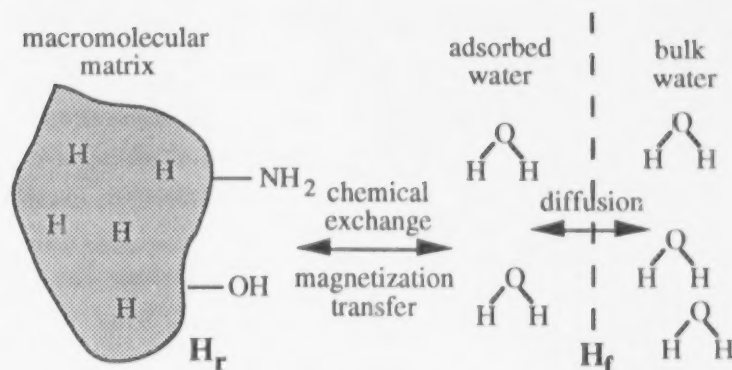


Figure 2.9 A pictorial representation of the interaction of water molecules with the macromolecular matrix (i.e. proteins, lipids, etc.) in tissue (adapted from Balaban 1992). The saturation by rf of the restricted macromolecular protons (H_r) can be transferred by magnetization transfer or chemical exchange to water adsorbed to the surface of the macromolecules. The adsorption water can then diffuse into the bulk water and the net magnetization of the free water protons (H_f) decreases from its equilibrium value.

Selective saturation of the macromolecular protons (H_r), with minimal direct saturation of the free water protons (H_f), is required to probe the magnetization transfer phenomena (Fig 2.10a). The rf saturation pulse (B_2) can be of two types, continuous wave or pulsed. Continuous wave (CW) saturation involves the application of a rf pulse with a weak amplitude and a long duration (Fig 2.10b). The long duration of the CW pulse allows the spin system to achieve a steady-state and yields a narrow frequency range for excitation of the spins. The frequency of the CW pulse is chosen to be far enough off-resonance from the water resonance ($\Delta\omega$) in order to saturate H_r selectively. The saturation is transferred throughout the resonance of the macromolecules by spin-diffusion (i.e. mutual flip-flops of adjacent spin-pairs). The reduction of the z-magnetization of the bulk water is monitored by the acquisition of the FID after a routine hard 90° rf pulse. Alternatively, the CW pulse can prepare the magnetization prior to an imaging sequence if spatial localization is required. The CW technique is simple to implement and is used for the MT measurements that are presented in this thesis. However, long CW pulses are not usually implemented on clinical MR scanners due to the additional hardware that is required, the limitations in available rf power, and the concern about unacceptably high power-deposition into

patients. Therefore, pulsed magnetization transfer experiments, which are readily programmable on clinical MR scanners and have no additional hardware requirements, have been developed that use binomial-, sinc-, or Gaussian-shaped rf pulses to saturate the very short T_2 components, such as the macromolecular protons, and not to saturate any long T_2 species, such as the bulk water (Hu 1992, Yeung 1992a, Schneider 1993, Pike 1993, McGowan 1994, Hurst 1995). We will not discuss pulsed MT methods any further since they were not used for our measurements.

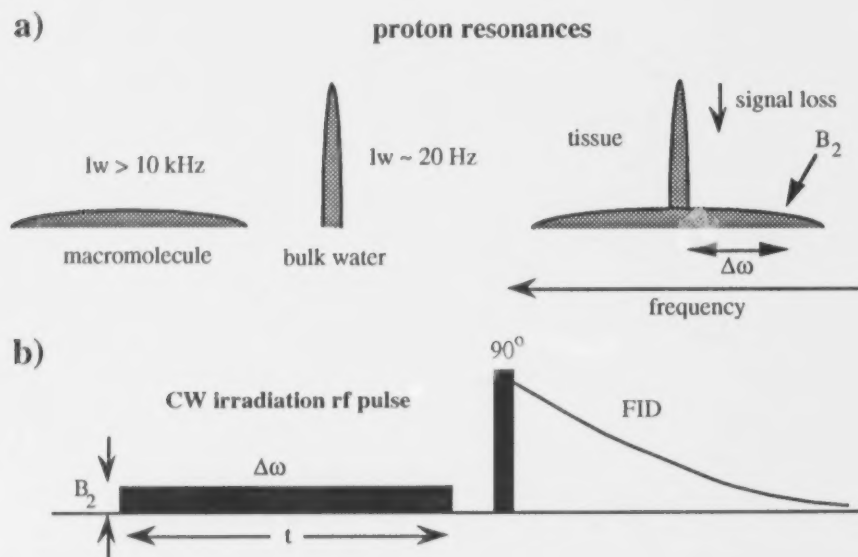
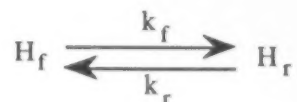


Figure 2.10 (a) The proton resonances of the macromolecular protons and the bulk water protons are illustrated (not to scale). The peak due to the macromolecules is not usually visible in a routine NMR spectrum since the linewidth (lw) of the peak is so large that the total signal is spread thinly over a large range of frequencies. In contrast, the lw of the bulk water protons is quite small. A CW rf pulse of strength B_2 is applied at an off-resonance frequency $\Delta\omega$. The CW pulse selectively saturates the macromolecules and the amplitude of the bulk water peak decreases if there is magnetization transfer between the macromolecules and the bulk water. (b) The CW pulse sequence for probing magnetization transfer is presented. The irradiation rf pulse saturates the macromolecules and the 90° pulse places the residual longitudinal magnetization of the bulk water into the transverse plane. The free induction decay (FID) is acquired, and is subsequently Fourier transformed to yield the residual amplitude of the bulk water.

In addition to the dipole-dipole interaction, magnetization transfer can occur as a result of chemical exchange between two sets of nuclei, say H_f and H_r ,



if the spin state of the nucleus does not change during the exchange. k_f and k_r are unidirectional rate constants. The Bloch equations for the longitudinal magnetization (Eqn. 2.21) can be modified to take chemical exchange into account (McConnell 1958),

$$\frac{dM_f}{dt} = R_{1f}(M_{of} - M_f) - k_f M_f + k_r M_r \quad [2.62a]$$

$$\frac{dM_r}{dt} = R_{1r}(M_{or} - M_r) + k_f M_f - k_r M_r \quad [2.62b]$$

where M is the magnetization of the protons, M_0 is the equilibrium magnetization, and R_1 is the longitudinal relaxation rate (inverse of T_1) in the absence of exchange. Forsén and Hoffman introduced a saturation transfer method of studying chemical exchange (Forsén 1963). Note that the following discussion of saturation transfer applies to dipolar coupling, as well as chemical exchange, since the effects of saturation transfer due to dipolar coupling and chemical exchange are very similar (Hoffman 1966). As we discussed earlier, one species, say H_r , is saturated *completely* (i.e. $M_r = 0$) and M_f is observed at steady-state (i.e. $dM_f/dt = 0$). The saturation of H_r is transferred to H_f by chemical exchange and, as a result, M_f decreases in magnitude. We assume negligible direct saturation of H_f (i.e. signal loss that is independent of MT). MT, on the other hand, is considered indirect saturation. Using the aforementioned conditions, the solution of Eqn. 2.62a yields the residual magnetization of H_f as a result of saturation (M_{sf}),

$$M_{sf} = \frac{R_{1f}}{R_{1f} + k_f} M_{of} \quad , \quad [2.63a]$$

or, alternatively, the degree of signal loss is

$$1 - \frac{M_{sf}}{M_{of}} = \frac{k_f}{R_{1f} + k_f} \quad [2.63b]$$

The rate constant, k_f , is proportional to the concentration of the restricted macromolecular pool. If R_{1f} is much greater than k_f , the degree of signal loss will be approximately proportional to the macromolecular concentration. However, if R_{1f} is comparable to or smaller than k_f , the degree of signal loss will no longer be proportional to the macromolecular concentration. Hence, in this latter case, the degree of MT measured at steady-state will not be as sensitive to the macromolecular composition as k_f , for example. The pseudo-first-order rate constant, k_f , can be determined by rearranging Eqn 2.63,

$$k_f = R_{1f} \{ -1 + (M_{of} / M_{sf}) \} \quad [2.64]$$

In order to calculate k_f , it is necessary to measure the spin-lattice relaxation time of the free protons in the absence of a contribution from chemical exchange (T_{1f}) (Mann 1977). T_{1f} can be calculated by measuring the T_1 of the H_f protons with an inversion recovery sequence as H_r is saturated (Alger 1984, Eng 1991). The T_1 measured in this manner is termed T_{1sat} or, in our case, T_{1fsat} for the free water protons, and it is related to R_{1f} ($= 1/T_{1f}$) and k_f via

$$\frac{1}{T_{1fsat}} = R_{1fsat} = R_{1f} + k_f \quad [2.65]$$

If there is MT, the observable T_1 of the H_f spins decreases upon irradiation of the H_r spins. The saturated macromolecular pool effectively behaves like the lattice and provides an additional pathway for relaxation of the free water spins. The substitution of R_{1f} (from Eqn. 2.65) into Eqn. 2.64 yields (Mann 1977)

$$k_f = \frac{1}{T_{1fsat}} \{ 1 - (M_{sf} / M_{of}) \} \quad [2.66]$$

Therefore, assuming complete saturation of the macromolecular protons, k_f can be estimated by observing the effects of irradiating H_r on both the T_1 and steady-state magnetization of the H_f spins. The measurement of k_f should supplement the measurement of the steady-state magnetization (M_{sf} / M_{of}) for assessing MT. k_f gives an idea of the approach to steady-state, as opposed to the steady-state level itself.

However, measuring k_f by this method assumes that the macromolecular pool is saturated completely. The saturation of H_f may be incomplete in some instances, and thus k_f can be referred to as an apparent rate constant, k_{app} . It follows that Eqn. 2.66 does not provide an accurate measure of the actual rate constant if the saturation is incomplete (Brooks 1994). Nonetheless, this simple approach of calculating k_{app} may still be useful for *relative* comparisons of MT in systems with incomplete saturation. An alternative and more complicated approach of calculating k_f involves curve-fitting procedures which do not require the assumption of complete saturation (to be briefly discussed later on in this chapter).

Another method of obtaining an estimate of the relative rapidity of approach to steady-state is to monitor the magnetization of H_f , M_{sf} , as a function of the duration (t_{irr}) of the irradiation rf pulse. Assuming instantaneous saturation of H_r , the solution of Eqn. 2.62a, with $R_{1f} = R_{1fsat} - k_f$ (i.e. Eqn. 2.65), yields (Forsén 1963, Eng 1991, Hajnal 1992a)

$$\frac{M_{sf}}{M_{of}} = \frac{T_{1fsat}}{T_{1f}} + k_{app} T_{1fsat} \exp(-t_{irr} / T_{1fsat}) \quad . \quad [2.67]$$

As the length of the irradiation pulse (t_{irr}) goes to infinity, the steady-state value of M_{sf} is

$$M_{sf} = M_{of} (T_{1fsat} / T_{1f}) \quad . \quad [2.68]$$

Also, note that, at steady-state, the proton spin-lattice relaxation time in the absence of chemical exchange (T_{1f}) is related to the T_1 obtained with concurrent saturation of H_r (T_{1fsat}). Upon rf irradiation, the magnetization attains its new equilibrium value with a time-constant T_{1fsat} . Therefore, in order to achieve steady-state, the duration of the saturation pulse should be at least $\sim 5 \cdot T_{1fsat}$. A more efficient transfer of magnetization would be expected to yield a shorter T_{1fsat} .

In addition to the indirect saturation via magnetization transfer from the macromolecular protons, signal loss of H_f can occur via direct saturation of the free water protons (i.e. often referred to as "rf bleedover" in the literature). This is not important with respect to understanding the interactions between H_f and H_r . However, it must be taken into account in order to not mistake direct saturation with

MT. The steady-state solution of the Bloch equations with relaxation terms yields the degree of direct saturation from the off-resonance irradiation rf pulse (Abragam 1961b),

$$\frac{M_{sf}}{M_{of}} = \frac{1 + T_2^2 (\Delta\omega)^2}{1 + T_2^2 (\Delta\omega)^2 + \gamma^2 B_2^2 T_1 T_2} \quad [2.69a]$$

$$= \frac{(1 / T_2^2) + (\Delta\omega)^2}{(1 / T_2^2) + (\Delta\omega)^2 + \gamma^2 B_2^2 (T_1 / T_2)} \quad [2.69b]$$

where M_{sf} is the steady-state magnetization after the irradiation pulse, M_{of} is the equilibrium magnetization with no irradiation pulse, γ is the magnetogyric ratio, T_1 and T_2 are the spin-lattice and spin-spin relaxation times, respectively, of the free water protons, B_2 is the strength of the irradiation pulse, and $\Delta\omega$ is the offset frequency of the rf from the H_f resonance. The difference between the degree of direct saturation and the observed signal loss results in a sole measure of the magnetization transfer (called the MT amplitude). The degree of direct saturation depends on the off-resonance frequency and strength of the saturation rf pulse and the T_1 over T_2 ratio of the free spins. The amount of direct saturation and hence, the degree of signal loss will be greater for smaller $\Delta\omega$, larger B_2 , and larger T_1 / T_2 .

The above discussion of MT has several shortcomings. The above model assumes complete saturation of the macromolecular spins and it does not consider the variation of MT (i.e. signal loss) as a function of the off-resonance frequency and B_2 strength of the rf pulse. Only a cursory overview of some of the more complicated and thorough models of MT will be presented because we did not use the following models to analyze the MT data from our samples of nerve. Theoretical two-pool (i.e. H_f and H_r) models of magnetization transfer that incorporate the frequency and strength of the rf have been described (Grad 1990, Wu 1991, Caines 1991a, Caines 1991b, Yeung 1992a, Holt 1994). Direct saturation of H_f is taken automatically into account (except for the models by Grad 1990 and Yeung 1992a). The liquid and semi-solid components are assumed to have Lorentzian lineshapes in these models. The Lorentzian lineshape is likely valid for the liquid components since the dipolar interactions are averaged out due to motions of the spins. However, the assumption of a Lorentzian lineshape is invalid for a rigid spin system like H_r since the dipolar

interactions are static. Hence, two-pool models of MT that use a Gaussian lineshape for H_r have been proposed and appear to provide better fits to the experimental data from model systems (Henkelman 1993, Yeung 1994, Kuwata 1994). A further extension is the use of a super-Lorentzian lineshape for the semi-solid pool (Morrison 1995a, Morrison 1995b). Curve-fitting (e.g. non-negative least-squares, simulated annealing) of the experimental data (M_{sf} / M_{of} versus $\Delta\omega$) to the theoretical models yields a set of optimized parameters that include relaxation times, sizes of the semi-solid pool, and magnetization transfer rate constants. In order to improve the accuracy of the fit, an extensive data set must be acquired at numerous offset frequencies and rf strengths. The acquisition of the large amounts of data required for these curve-fitting procedures is generally feasible in model systems, yet it is unrealistic for routine in-vivo measurements in humans.

2.2 Some Applications of NMR to the Study of Water in the Nervous System

Section 2.1 dealt with some of the theoretical and experimental aspects of NMR. In particular, some of the properties that can be measured by NMR were introduced and they included nuclear relaxation (T_1 and T_2), diffusion, and magnetization transfer. A background of the aforementioned properties in the nervous system is presented in this section. Only a very brief overview of relaxation is presented since the focus of this thesis is diffusion and, to a lesser extent, magnetization transfer. The overview will consist of both basic research (e.g. measurements in excised tissue and animal models of disease) and clinical studies. Once again the discussion is limited to those aspects which are directly relevant to this thesis. In addition, we introduce the problems which are addressed in this thesis.

2.2.1 Nuclear Relaxation of Water Protons in the Nervous System

The tissue contrast available in routine MRI is due primarily to differences in the T_1 and T_2 relaxation times of the water protons in normal and pathological tissue. The physical reason behind the differences in the relaxation times is thought to involve the unique composition of macromolecules, or lack thereof, in each tissue. For example, T_1 - and T_2 -weighting allows excellent discrimination between gray (GM)

and white matter (WM). The presence of myelin is postulated to be the source of the decreased relaxation times of WM with respect to GM (Koenig 1990).

The analysis of relaxation in tissue is complicated by the fact that tissue is comprised of multiple water compartments. For example, the inter-axonal space (e.g. the space between the exterior of the myelin sheaths in adjacent myelinated axons), the axonal space, and myelin are three identifiable water compartments in white matter. There is no *a priori* reason to believe that each water compartment will have the same relaxation characteristics. Due to the spatial averaging over an entire voxel of tissue, the NMR signal is usually a sum of signals from all of the water compartments. Therefore, changes in one specific water compartment (e.g. extracellular edema, myelin loss, etc.) are not readily apparent if the NMR signal is averaged spatially. In an attempt to improve the specificity of MRI in the diagnosis of disorders of the nervous system, careful measurements and analyses of the T_1 and T_2 relaxation decay curves of nerve tissue have been performed in this and other laboratories.

The longitudinal relaxation decay curves were found to be monoexponential (i.e. one component) in the gray and white matter of cat brain (Menon 1991), and in the ventral nerve cord of the crayfish (Menon 1992). Presumably, the exchange of water between the compartments is fast enough, on the T_1 time-scale, to average the signal from the various compartments and yield a single T_1 value. Therefore, T_1 does not provide any direct information about the compartmentalization of water in the nervous system.

In contrast to longitudinal relaxation, the transverse relaxation of water protons is often multi-exponential in the white matter of brain, in spinal cord, and in nerve, both in-vitro and in-vivo. The transverse relaxation decay curves obtained for these tissues are fitted generally with three components (sometimes two), namely, a long transverse relaxation time (T_2) component ~ 200 -600 ms, an intermediate T_2 component ~ 70 -200 ms, and a short T_2 component ~ 10 -50 ms (Table 2.1). These multiple T_2 components are frequently interpreted in terms of distinct water pools within the heterogeneous tissue. For example, the long, intermediate, and short T_2 components observed in the aforementioned tissues of the nervous system are usually assigned to water in the inter-axonal, axoplasmic, and myelin domains, respectively. Because of its relative lack of macromolecules, the inter-axonal (extracellular) water is

expected to have a T_2 most similar to bulk water. Water in myelin, on the other hand, is expected to have a very short T_2 because of its high concentration of macromolecules. The T_2 of axoplasm is anticipated to possess intermediate values. The assignment of the T_2 components to individual water compartments assumes that the exchange between the three water compartments is slow on the T_2 time-scale.

Table 2.1 A summary of the long, intermediate, and short transverse relaxation time (T_2) components observed for water protons in white matter, spinal cord, and nerve.

Sample	Long		Intermediate		Short		Reference
	T_2 (ms)	%	T_2 (ms)	%	T_2 (ms)	%	
garfish olfactory nerve ^a	510	21	170	77	---	--- ^c	Fenrich 1992
garfish trigeminal nerve ^a	460	42	140	40	34	14	Fenrich 1992
garfish optic nerve ^a	480	25	140	53	49	16	Fenrich 1992
crayfish abdominal nerve ^a	600	32	200	59	50	7	Menon 1992
frog sciatic nerve ^a	310	21	70	50	17	29	Vasilescu 1978
frog sciatic nerve ^a	271	37	76	40	20	24	Does 1995a
frog sciatic nerve ^b	247	44	71	29	22	27	Does 1995a
guinea pig spinal cord ^a	215	30	76	57	10	13	Stewart 1993
guinea pig white matter ^a	---	--- ^c	92	96	10	4	Stewart 1993
cat white matter ^a	306	3	89	86	13	7	Menon 1991
human white matter ^b	207	19	74	73	18	8	Kreis 1992

^a Measurements on samples in-vitro.

^b Measurements on samples in-vivo.

^c Component was not observed.

There is considerable evidence supporting the hypothesis of water compartmentalization as the source of the multi-exponentiality in the transverse relaxation decay curves of water in ordered axonal systems. First, in T_2 -weighted microimages of the crayfish abdominal nerve cord, giant axons appeared hypointense in relation to their surroundings, suggesting that the T_2 (axoplasm) < T_2 (extracellular and glia) (Menon 1992). Second, the presence of a short T_2 component in two

myelinated garfish nerves, but absent in a nonmyelinated garfish nerve suggested that the short T_2 component corresponded to myelin water (Fenrich 1991, Fenrich 1992). Third, the amplitude of the short T_2 peak decreased as the degree of demyelination increased in the white matter and spinal cord of guinea pigs induced with experimental allergic encephalomyelitis, further supporting the short T_2 component assignment (Stewart 1993). In addition, the short T_2 component seen in frog sciatic nerve, *in vivo*, disappears with progressive nerve degeneration and concomitant myelin loss (Does 1994, Does 1995a). The frog sciatic nerve also loses its multi-exponential character upon degeneration. Fourth, short T_2 component maps in human brain *in vivo* correspond well with known distributions of myelin within the brain (MacKay 1994). Consequently, the multiple T_2 components likely correspond to individual compartments of water. Even if this hypothesis proves to be incorrect, the presence of *intact* axons and myelin seems to be a prerequisite for the multi-exponentiality of the transverse relaxation decay curve.

Therefore, alterations in the microstructure of the nerve, spinal cord, or white matter may be reflected in the transverse relaxation decay curve. The potential uses of multi-exponential T_2 analysis include the monitoring of myelin loss in multiple sclerosis lesions (MacKay 1994) and following the progression of degeneration in peripheral neuropathies (Does 1995a). The presence of the long T_2 component in peripheral nerve allows severe T_2 -weighted images (*i.e.* use long echo-time) to delineate nerve from the surrounding muscle quite clearly (Does 1995b).

2.2.2 Water Diffusion in the Nervous System

In the late 1960's and early-to-mid 1970's there was great interest in understanding the state of ions and water in cytoplasm. Studies of nuclear relaxation in excised tissue had shown substantial differences between water in tissue (where $T_1 > T_2$) and pure water (where $T_1 \sim T_2$) (Cope 1969). This difference was attributed to a decreased mobility and an increased "ordering" of some of the water in the tissue. To provide a *direct* measure of particle mobility in tissue, measurements of diffusion were performed. If the diffusion time is small enough to minimize the interactions of water with cell membranes, the diffusion coefficient depends on the intrinsic viscosity of the cytoplasm (and other water compartments) and on the obstruction of diffusing

water molecules by structures within the cell. As the diffusion time is increased, diffusing water will encounter more barriers in the form of membranes. It is often difficult to separate the contributions of the intrinsic viscosity and the obstruction effect of barriers to the decrease of $D(H_2O)$. The diffusion coefficients of ions and water in tissue are reduced typically by a factor of 2-5 in comparison to diffusion coefficients obtained in bulk water. Many of the early measurements were made in excised muscle (Kushmerick 1969, Finch 1971, Chang 1973, Cleveland 1976, Tanner 1979), but there were also some measurements of diffusion in excised brain tissue (Hansen 1971). It was shown that the translational diffusion of water in brain is hindered to a greater extent than in muscle.

The advent of diffusion-weighted imaging (DWI) in the mid-1980's yielded the possibility of measuring diffusion coefficients in various tissues *in-vivo*. Therefore, a resurgence of diffusion studies swept over the magnetic resonance community in biology and medicine. The utility of DWI for improving contrast and providing new information about healthy tissue and about numerous disorders of the nervous system was studied extensively.

Early measurements of water diffusion in normal human white matter (WM), *in-vivo*, showed large variations of D_{NMR} (Thomsen 1987). They proposed that the regional differences in diffusion could be due to anisotropic diffusion in the white matter tracts. In other words, the water molecules would preferentially diffuse along the length of the axons and would be hindered by barriers such as the myelin sheath when diffusing perpendicular to the axons. Therefore, depending on the relative orientation of the applied gradients and the white matter tracts, a spread of D values could be measured in WM. Gray matter, on the other hand, does not have an oriented fiber structure and thus would not be expected to exhibit anisotropic diffusion. Others have observed a directional dependence of D in the WM of human brain *in-vivo* (Chien 1988, Chien 1990, Chenevert 1990, Turner 1990b, Doran 1990). An interesting study of anisotropic water diffusion in normal WM was presented by Moseley et al. (Moseley 1989, Moseley 1990a). Diffusion was confirmed to be anisotropic in the WM of cat brain, *in-vivo*, whereas diffusion was isotropic in the gray matter. Furthermore, anisotropy was observed in the white matter of cat spinal cord (Moseley 1990a) and in excised myelinated nerves from rabbits (Segawa 1992a). The observation of significant anisotropy in excised nerve helped to alleviate any fears of

anisotropy being due to pulsatile motions in-vivo. A summary of diffusion coefficients measured parallel $\{D_{\text{NMR}}(\parallel)\}$ and perpendicular $\{D_{\text{NMR}}(\perp)\}$ to the length of the axons in white matter is presented in Table 2.2. The anisotropy is quantified by the anisotropic diffusion ratio which is given by $D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)$. An anisotropic diffusion ratio of ~ 1 would indicate isotropy. D values for gray matter are provided in Table 2.2 for comparison. As a quick aside, we should note that anisotropic diffusion is also observed in muscle, the degree of anisotropy ($\sim 1.3 - 1.4$) being much less than that observed in white matter (Cleveland 1976, Moseley 1991b, Garrido 1994).

Table 2.2 Diffusion coefficients measured parallel ($\parallel$) and perpendicular ($\perp$) to the length of the axons (for white matter) and the anisotropic diffusion ratio of water in normal gray matter and white matter in-vivo ^a are presented.

Sample	$D_{\text{NMR}}(\parallel)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$	Reference
<i>Gray matter</i> ^b				
cat	0.67	0.69	1.0	Moseley 1990a
human	0.67	0.67	1.0	Turner 1990b
human frontal	0.86	0.84	1.0	Le Bihan 1993
<i>White matter</i>				
cat corpus callosum	1.3	0.35	3.7	Moseley 1990a
cat corona radiata	1.0	0.45	2.2	Moseley 1990a
human	1.0	0.43	2.3	Turner 1990b
human	0.5	0.3	1.7	Merboldt 1992
human temporal	1.03	0.38	2.7	Douek 1991
human frontal	0.92	0.34	2.7	Douek 1991
human corpus callosum	1.2	0.4	3.0	Horsfield 1994
human optic radiation	1.25	0.48	2.6	Sakuma 1991
human frontal lobe	1.24	0.48	2.6	Sakuma 1991

^a Temperature of samples is 37 °C.

^b Gray matter does not have an oriented fiber structure and therefore $\parallel$ and $\perp$ refer to diffusion measured in two orthogonal directions that are not necessarily $\parallel$ and $\perp$ to any particular structure.

The anisotropic diffusion of water in white matter / nerve has been used to map the myelin fiber orientations in brain noninvasively (Douek 1991, Coremans 1994) and to improve the visualization of peripheral nerve (Howe 1992). The anisotropy is no doubt related to the ordered arrangement of the myelin fibers. However, little has been done to determine the relative contributions of the various structural components of white matter to the anisotropy of the water diffusion coefficients. Nonetheless, several possible origins of anisotropy have been postulated. For example, the myelin sheath, the axonal membrane, and the neurofibrils (neurofilaments, microtubules) are three longitudinally oriented structures that could impart nonrandom barriers to diffusion (Fig. 2.11), and hence reduce $D_{\text{NMR}}(\perp)$ with respect to $D_{\text{NMR}}(\parallel)$ (Le Bihan 1991). Alternatively, the diffusion parallel to the length of the axons could be accentuated by axonal transport. Others have suggested that water diffusion, as measured by NMR, could be anisotropic due to local susceptibility-difference-induced gradients in the nerves and white matter (Hong 1992, Lian 1994). However, the preferred, but unproven, *hypothesis* of many groups has been the hindering of perpendicular water diffusion by the presence of a myelin sheath.

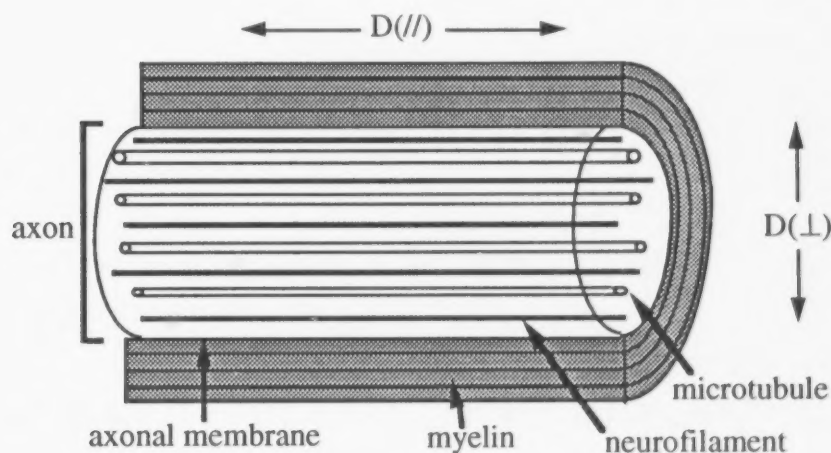


Figure 2.11 A simplistic schematic of the longitudinal view of a myelinated axon. Myelin, the axonal membrane, microtubules, and neurofilaments are longitudinally-oriented structures that could hinder water diffusion perpendicular to the axon and cause $D(\parallel)$ to be larger than $D(\perp)$ (i.e. anisotropic diffusion).

A good portion of this thesis (Chapter 4) is devoted to assessing the potential sources of anisotropic diffusion in nerve and white matter. To evaluate whether myelin is a necessary prerequisite for anisotropic diffusion, we measured water diffusion in the nonmyelinated olfactory nerve and, for comparison, the myelinated trigeminal and optic nerves of the garfish which are used as simplified models of white matter. A q -space analysis of the diffusion decay curves obtained from the garfish nerves are also presented in Chapter 4. In addition, the garfish nerves were treated with vinblastine in order to depolymerize the microtubules and inhibit fast axonal transport. This permitted the role of fast axonal transport and microtubules for generating anisotropic water diffusion to be evaluated. Furthermore, to confirm that anisotropy decreases upon structural degradation of the nerve, diffusion measurements were made in excised garfish nerves that were allowed to degenerate post-excision at low temperature for 2 months. The giant axon of the squid has a large enough diameter so that very few water molecules, relatively speaking, will interact with the axonal membrane over typical NMR diffusion measurement times. Hence, measurements of diffusion in the squid giant axon tested the ability of the neurofilamentary cytoskeleton to inhibit water diffusion perpendicular to the axon. The influence of local susceptibility-induced-gradients (called local gradients) on anisotropic diffusion was evaluated using two methods to eliminate the background gradients. First, since local gradients are only present when the nerves are aligned perpendicular to the static magnetic field (B_0), we measured diffusion in nerves (garfish and frog sciatic) aligned parallel to B_0 . Second, the effect of the local gradients on the measured diffusion coefficients in the garfish and frog nerves was eliminated by using a bipolar gradient pulse sequence to measure diffusion. The results from these experiments and their implications are presented and discussed in Chapter 4. Evaluating the influence of various cellular structures on water diffusion will allow us to understand and to predict changes of water diffusion in normal and pathological tissue.

As we mentioned in Section 2.2.1, the multiple T_2 components observed in normal nerve tissue are believed to correspond to individual water compartments in the nerve (i.e. axonal, inter-axonal, and myelin (when present)). The magnetization from shorter T_2 components can be reduced in amplitude or even eliminated by moving to longer times along the transverse relaxation decay curve. Therefore, placing a CPMG sequence with sufficient 180° refocussing rf pulses in front of a spin-echo diffusion sequence should allow a measurement of the diffusive abilities of the long T_2 component. Chapter 5 describes an attempt to measure the diffusion coefficients of water in the various compartments, particularly the inter-axonal compartment, in normal garfish nerve.

Large areas of white matter in neonates are nonmyelinated or myelinated to a much lesser extent than in adults. Typically, T_1 - and T_2 -weighted imaging is used to follow the development of myelination. Further, anisotropic diffusion in white matter is hypothesized to be related to the degree of myelination. To test this hypothesis, measurements of diffusion in neonate and infant brain were compared to those in adult brain (Nomura 1990, Sakuma 1991, Rutherford 1991). The anisotropic diffusion ratio (i.e. $D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)$) increased as the white matter matured (Sakuma 1991). The difference was due to a decrease in $D_{\text{NMR}}(\perp)$ with maturation whereas $D_{\text{NMR}}(\parallel)$ remained relatively constant. Areas of brain showing anisotropic diffusion agreed with areas of myelination, as indicated by T_1 -weighted images, in the infants and adults. In neonates, anisotropy was not observed generally in regions of white matter that did not indicate myelination. However, there were exceptions where anisotropic diffusion was seen in regions of brain that did not demonstrate myelination on the T_1 - and T_2 -weighted images (Nomura 1990, Rutherford 1991). The authors in these studies interpreted this finding by suggesting that anisotropic diffusion was capable of detecting myelination earlier than T_1 -weighted imaging. However, the presence of structural factors in the immature white matter, other than myelin, that are able to generate anisotropic water diffusion could be an alternative interpretation. In fact, anisotropic water diffusion was observed in the white matter of rat pups prior to any histological evidence of myelin (Wimberger 1995). They concluded that anisotropic water diffusion must not be regarded as myelin specific.

Nevertheless, there have been several studies that evaluated the utility of diffusion-weighted imaging (DWI) for the characterization of demyelination in multiple

sclerosis (MS) plaques (Mikulis 1988, Forti 1991, Larsson 1992, Segawa 1992b, Christiansen 1993, Heide 1993). Unfortunately, these studies have been inconclusive. Intuitively, one would expect an increase in D and a decrease of anisotropy within a lesion with progressive demyelination and edema. The diffusion coefficient of water has been found to be quite variable (i.e. larger, smaller, or normal) in MS lesions (that have been identified previously by T_1 - or T_2 -weighted imaging). In some instances, the lesions showed significant anisotropic diffusion (Forti 1991, Segawa 1992b). Therefore, at this present time, DWI does not appear to be useful in evaluating the condition of MS plaques.

There have been cases in which DWI has been useful in detecting axonal damage. Anisotropy decreased from its value of 2.4 in normal individuals to 1.5 in lesions of the pyramidal tract of patients (Segawa 1993). $D_{\text{NMR}}(\parallel)$ remained constant and $D_{\text{NMR}}(\perp)$ increased significantly in the lesion, presumably due to the loss of myelin. However, since these findings were in-vivo, comparisons with histology were not possible. Therefore, any explanations for the changing D values are purely speculative. Reductions in anisotropy were observed in the lateral and dorsal white matter of injured spinal cords from rat (Ford 1994). Unfortunately, their model involved fixing the samples of spinal cord, the fixation process having unknown effects on water diffusion. A model that does not require fixation of the sample would be preferred. In order to confirm and improve upon these studies of anisotropy in systems with axonal degeneration, measurements of water diffusion in an in-vivo model of Wallerian degeneration in the sciatic nerve of the frog were performed. The sciatic nerve samples were not fixed after excision. Changes in the diffusion coefficients were related with structural features observed in light and electron micrographs of the damaged nerves. The results of our experiments on degenerated nerve are presented in Chapter 6.

One of the most promising frontiers for DWI is its use for the detection and evaluation of ischemic injury. Using a middle cerebral artery occlusion model of acute stroke in cat brain, Moseley et al. first showed that DWI could be used to detect ischemic events much earlier than conventional T_2 -weighted imaging (Cohen 1989, Moseley 1990b, Moseley 1990c). The area of ischemia (in the gray matter) showed decreased diffusion coefficients (~ 30 -50% lower than normal values) within 1 hour of the onset of ischemia. Therefore, the water protons in the ischemic region do not lose

as much signal and the ischemic region appears hyperintense in the MR image. In fact, the hyperintensity can be reversed if the ischemic region is reperfused (Mintorovitch 1991). Also, at later stages of the ischemia (48 hours after onset) with no reperfusion, the diffusion coefficient increases back to its normal value (Knight 1991). The exact nature of the reduction of D is unknown at this present time. It was suggested that the decreased D values of water could be due to intracellular swelling (cytotoxic edema), the intracellular environment presumably having a greater restrictive influence on diffusion than the extracellular environment.

Since Moseley et al.'s first paper on the use of DWI for detecting early focal ischemia, there has been literally an international explosion of research on this topic. Studies from other groups, including DWI studies of cerebral infarction in humans, quickly confirmed and expanded upon the findings of Moseley et al. (MacFall 1990, Moonen 1991, Knight 1991, Busza 1992, Chien 1992, Finelli 1992, Minematsu 1992a, Minematsu 1992b, Warach 1992). In contrast to acute stroke, chronic (i.e. old) stroke shows increases in the diffusion coefficient, presumably due to vasogenic edema and cellular disruption (Chien 1992, Takahashi 1993). Decreased diffusion of water in brain has been observed in models of global ischemia (Bizzi 1993, Hossmann 1994) and thromboembolic stroke (de Crespigny 1993). The reduction of D is not limited to gray matter since D is also reduced in ischemic white matter (van Gelderen 1994b, Dardzinski 1993, Warach 1995). The use of the trace of the diffusion tensor has been proposed to remove the effects of anisotropic diffusion on DWI and to improve the visualization of ischemic regions (van Gelderen 1994b, Mori 1995). Others have shown that the ischemic region can be subdivided according to quantitative measures of the diffusion coefficient, thus yielding a potential means of distinguishing reversible from irreversible ischemic injury (Dardzinski 1993). More rapid means of monitoring diffusion have shown that D decreases significantly within 5-10 minutes of the onset of ischemia in a cat (Davis 1994). After reperfusion, D increases to its normal value quite rapidly (Davis 1994). DWI can also differentiate ischemic tissue from traumatized tissue (Hanstock 1994). Recent studies have shown that ischemic regions of human brain are clearly delineated in diffusion-weighted MR images, the deleterious effects of patient motion being alleviated by ultrafast echo-planar imaging (EPI) (Warach 1995) or by corrective navigator-echo techniques (de Crespigny 1995).

The basis of the reduction of D in ischemic tissue, as compared to normal tissue, is unknown. Possible explanations are cytotoxic edema (function of energy failure), decreased temperature, and reduced pulsatile motions. A decrease in the cell membrane permeability is another potential reason (Helpert 1992). However, it appears that the permeability of the membrane does not likely play a role in the reduction of D in ischemic tissue (Latour 1994a, Szafer 1995). On the other hand, changes in the cellular volume fraction appear to be important (Latour 1994a, Szafer 1995). Experimental studies have shown that cytotoxic edema (i.e. influx of extracellular water into the intracellular space) can cause a decrease, to some extent, of the measured diffusion coefficient (Benevise 1992, Sevick 1992, Ebisu 1993). Other studies have related the reduced D value with energy failure (and arrest of the Na^+/K^+ pump) within the tissue (Busza 1992, Mintorovitch 1994, Hossmann 1994). Diffusion-weighted MR images have also been compared to histopathology (Pierpaoli 1993, Knight 1994). Regions of ischemic damage corresponded to regions of change in the diffusion-weighted images. We should note that any information about the influence of cellular structures on water diffusion in our nerve samples will contribute to the understanding of diffusion in normal nervous tissue and perhaps to a better idea of the basis of the reduction of diffusion in ischemic tissue.

Diffusion-weighted imaging provides a non-invasive, quantitative method of monitoring the extent of ischemic damage shortly after the onset of ischemia. Also, DWI may allow discrimination between reversible and irreversible damage. Therefore, the impact of various therapies can be monitored at times when they are expected to have the most protective influence. There have been several reports of the assessment by DWI of the effectiveness of various pharmacological neuroprotectants (Kucharczyk 1991, Minematsu 1993, Lo 1994, Kozniowska 1995). Furthermore, the effect of hypothermia on focal ischemia was evaluated by DWI (Jiang 1994). In summary, the following quote by Warach et al. stresses the potential impact of this new technology for the investigation of stroke, "*we predict that the impact of whole-brain EPI DWI in the diagnosis of cerebral infarction may come to be viewed as analagous to the introduction of electrocardiography for the diagnosis of myocardial infarction, i.e., a rapid, reliable, objective, accurate, and essential emergency diagnostic test that will guide the development and application of acute therapeutic interventions*" (Warach 1995).

Furthermore, MR measurements of water diffusion in tumours have proven useful in differentiating various tumour components (Tsuruda 1990, Chenevert 1991, Tien 1994). For example, water in solid components of tumours possessed smaller D values than water in cystic or necrotic portions of the tumour. Therefore, DWI may allow better characterization of tumours, especially when their appearances are similar on T_1 - or T_2 -weighted images. MR imaging of water diffusion may be of use in the evaluation of other neurological disorders (Latour 1994b, Zhong 1995). Our discussion, so far, has focussed on the diffusion of water. However, it is possible to measure the diffusion coefficients of metabolites (e.g. N-acetyl-aspartic acid, creatines, cholines) in brain in-vivo (Merboldt 1993).

2.2.3 Magnetization Transfer in the Nervous System

Magnetization transfer (MT) has proven to be a useful contrast mechanism, in some instances, in MRI. Furthermore, MT may also help to characterize tissue. Often, the amount of signal loss due to MT is quantified by the magnetization transfer ratio (MTR). The MTR is given by $\{(M_0 - M_s)/M_0\}$ where M_s and M_0 refer to the amplitude of the water proton magnetization with and without application of the irradiation radiofrequency (rf) pulse, respectively (Fig. 2.10). The loss of proton signal from the bulk water upon selective rf irradiation of the macromolecular protons is dependent upon a dipolar interaction and/or chemical exchange with protons of the macromolecular matrix (Fig. 2.9). Therefore, the degree of signal loss due to MT will depend upon the amount and type of macromolecules present in the tissue. For example, fat and cerebrospinal fluid (CSF) do not lose any signal due to MT, in contrast to muscle and brain that lose a significant amount of signal. In fact, the first description of off-resonance rf irradiation to generate improved contrast between fat and muscle in limbs was presented by Paul Lauterbur's group in 1983 (Muller 1983). However, the widespread interest in in-vivo imaging applications of MT did not surface until the report in 1989 by Wolff and Balaban (Wolff 1989).

MT can improve the contrast between gray and white matter, as well as the contrast between CSF and the surrounding tissue. The degree of signal loss in brain is significant and is *generally* greater in normal WM than in normal GM (Table 2.3). However, there are many instances in which the MTR's of GM and WM are reported

to be quite similar. Finelli et al. have shown that GM and WM can appear isointense given the appropriate rf parameters of the MT pulse (Finelli 1994). Due to different types of irradiation rf pulses and their parameters (e.g. off-resonance frequency ($\Delta\omega$) and rf strength (B_2)), there is a fair amount of variability in the MTR's of GM and WM (Table 2.3). Also, at smaller off-resonance frequencies, the signal loss may be due to direct saturation of the bulk water, as opposed to indirect saturation via MT with the macromolecular protons. Many of the reported MTR's are not likely corrected for direct saturation. The dependence of the degree of signal loss on the rf strength and off-resonance frequency for the continuous wave (CW) irradiation technique has been documented for white matter (Henkelman 1994). However, given a particular MT protocol, the MT technique is very reproducible and the standard deviations of the MTR's are usually quite small ($< 3\%$) for normal tissue.

The loss of signal due to MT is not instantaneous. It takes time (hundreds of milliseconds to seconds) for the macromolecule/water system to reach a steady-state in which further application of irradiation rf has no further effect on the observed signal amplitude. WM appears to reach steady-state more quickly than GM (Hajnal 1992a, Kurki 1995) which indicates a more efficient transfer of magnetization between the macromolecular protons and the water protons in WM.

Table 2.3 Magnetization transfer ratios (MTR) ^a for normal white (WM) and gray matter (GM).

Sample	WM MTR (%)	GM MTR (%)	Length and type of MT pulse / B ₂ (μT) / Δω (kHz)	Reference
human	42	39	19 ms sinc / 3.7 / 2	Dousset 1992
guinea pig	42	----	19 ms sinc / 3.7 / 2	Dousset 1992
human	30 - 33	23-24	64 ms sinc / ? / 2	Gass 1994
human	40 - 45	----	300 ms ? ^b / 0.35 / 7.2	Tomiak 1994
cat	41 - 43	36 - 37	19 ms sinc / 3.7 / 2	Lexa 1994
human	59	53	1 ms binomial / 23 / -- ^c	Pike 1993
human	37	----	10 ms sinc / 21 / 1	Finelli 1994
human	40 - 45	36 - 39	300 ms ? ^b / 0.35 / 7.2	Prager 1993
bovine	62	----	5 s CW / 16 / 7.5	Kucharczyk 1994
bovine	54	53	7 s CW / ~ 16 / ~ 10	Morrison 1995a
human	60	52	~ 1 s CW / 17 / 5	Hajnal 1992a
human	43	42	500 ms ? ^b / ? ^b / 8	Lundbom 1992
human	63	62	650 ms Gaussian / 25 / 4	Kurki 1995

$$^a \text{MTR (\%)} = \frac{(M_0 - M_s)}{M_0} 100.$$

^b The type or strength of MT pulse is not evident in the manuscript.

^c Binomial pulses are usually applied on-resonance for MT.

The source of the difference in the MT characteristics between WM and GM is unknown. The difference is usually *assumed* to be due to the presence and relative absence of myelin in WM and GM, respectively. Therefore, the higher membrane content of white matter may be responsible for the differences in the relaxation and MT characteristics of WM and GM (Fralix 1991). There have been several investigations of the MT abilities of various molecular components, particularly lipids, of white matter. The presence of surface hydroxyl or amine groups are important for MT (Ceckler 1992). Previous measurements suggested that sphingomyelin and cholesterol in lipid bilayers (suspended in unbuffered water) played major roles in the MT of white matter (Fralix 1991, Koenig 1991, Ceckler 1992). Recent studies of MT in multilamellar vesicles (suspended in physiological buffer) made of phosphatidylcholine with varying proportions of cholesterol, sphingomyelin, and

galactocerebrosides (abundant components of myelin) (Kucharczyk 1994) have disputed the results of the earlier studies by Fralix et al., Koenig, and Ceckler et al. The main findings of this paper by Kucharczyk et al. were as follows. The MT of the vesicles with galactocerebroside was much greater than those with either cholesterol or sphingomyelin, implying that galactocerebroside is the lipid that most likely explains the increased MT and relaxation properties of white matter. Cholesterol and sphingomyelin were less MT active when the vesicles were suspended in physiological buffer (pH ~ 7) than when they were suspended in unbuffered water (pH ~ 5). The importance of galactocerebroside to MT is corroborated by the fact that galactocerebroside has a larger number of hydroxyl groups than cholesterol or sphingomyelin and that there is a greater difference in the concentration of galactocerebroside between WM and GM. The dependence of MT on the pH of the suspension suggested that chemical exchange between the protons of the macromolecules and the bulk water plays a substantial role in MT (Fig. 2.9). Therefore, MT in tissue does not occur as a sole result of dipolar coupling.

Despite the apparent differences in MT between GM and WM, MT does not appear to be particularly tissue specific. The MT curves (i.e. signal amplitude as a function of the off-resonance frequency of the irradiation rf pulse) are quite similar for quite a range of tissues such as muscle, white matter, gray matter, optic nerve, liver, tendon, cartilage, and kidney (Henkelman 1994, Morrison 1995a). This implies a similar structure for the rigid macromolecular pool.

To further investigate the contribution of myelin to the MT properties in WM, careful measurements of MT in *normal* nonmyelinated and myelinated nerves were performed and the results are described in Chapter 7. The nonmyelinated olfactory nerve and the myelinated trigeminal and optic nerves of the garfish were used as our simplified models of white matter. Both the steady-state levels of signal loss and the rapidity of approach to steady-state were examined using continuous-wave irradiation at several rf strengths.

The utility of magnetization transfer contrast (MTC) has been tested for several applications. By greatly attenuating the signal from the stationary background brain tissue, MTC, in combination with MR angiography, substantially enhances the visualization of small blood vessels in the brain (Pike 1992, Edelman 1992). Ordidge

et al. have shown that MT is lower in ischemic brain than in normal brain, presumably due to breakdown of cellular structure in the ischemic brain (Ordidge 1991). Others have reported that MTC could distinguish between acute infarcts and chronic infarcts (Prager 1993). MTC demonstrated white matter abnormalities in patients with metastatic lesions whereas the abnormalities were not visualized with T₂-weighted MRI (Boorstein 1994). Also, Lundbom et al. found that tumours with fluid or cystic components showed lower MTR's than in solid tumours (Lundbom 1992).

The application of MTC to the study of demyelinating lesions in patients with multiple sclerosis (MS) has received the most interest. One of the main limitations of routine MRI is its lack of specificity. For example, edematous lesions without myelin loss and demyelinated lesions could appear similar (i.e. hyperintense) on T₂-weighted images. An excellent study by Dousset et al. of experimental allergic encephalomyelitis (EAE) (an experimental model of acute MS) and MS lesions showed that MT may be able to improve the specificity of MR imaging (Dousset 1992). EAE lesions, which are primarily edematous with no myelin loss, had higher MTR's than MS lesions which are primarily demyelinated. The lower MTR in the MS lesions reflects a reduction in the amount of macromolecular components. Furthermore, there was a wide range of MTR's (~ 36%) for the MS lesions, reflecting lesions with different age and stages of degeneration. In comparison, MTR in normal WM only shows a range of ~ 5%. The decrease of MT in MS lesions was confirmed by others (Gass 1994, Tomiak 1994). Also, MT imaging of MS patients permitted the visualization of lesions that were not previously visible by routine T₁-weighted imaging (Finelli 1994). Others have correlated an increase in "marker peaks" (potential myelin breakdown products) observed with proton spectroscopy with decreased MTR values observed in the same MS lesions (Hiehle 1994). They concluded that MT is sensitive to myelin loss. However, we must state that the contribution of axonal proteins to MT is unknown, and hence, it is possible that, in some instances, a decrease in the MT of WM may have a significant contribution from the structural degradation of the axons rather than from sole degradation of the myelin.

Interestingly, magnetization transfer contrast (MTC) has been used clinically by the Radiology Department at the University of Alberta Hospitals for the past eighteen months. Both on- and off-resonance MT rf pulses are available for presaturation. Apparently, there is some controversy amongst the radiologists as to

whether or not MTC actually improves diagnosis of disease. However, some find it useful. MTC is often used in augmentation studies to improve the conspicuity of a lesion by suppressing the background signal from normal brain tissue. MTC can be used with Gd enhancement to further improve contrast. MTC has enhanced the detection of demyelinating lesions and improved the visualization of blood vessels in MR angiography.

CHAPTER 3

Experimental Materials and Methods

This chapter is subdivided into three major parts. Section 3.1 describes the animal models (garfish, squid, frog) used in our experiments. The reasons for choosing the particular animal model, the dissection procedures of the nerves, the storage buffer, validation of nerve integrity, etc. are discussed as well. Section 3.2 describes the hardware and pulse sequences that were used in our measurements of diffusion. Further, the validation of our diffusion techniques on phantoms with known diffusion coefficients is presented. Section 3.3 describes the measurement techniques that were used in our studies of magnetization transfer, as well as measurements of transverse and longitudinal relaxation.

3.1 Animal Models

All dissection procedures were approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

3.1.1 Garfish

3.1.1.1 Description and Source

The garfish is a prehistoric, olive coloured fish with tough scales, a long snout, and very sharp teeth as its most prominent features (Fig. 3.1). Three types of garfish were investigated: the spotted garfish (*Lepisosteus oculatus*), the long-nosed garfish (*Lepisosteus osseus*), and the short-nosed garfish (*Lepisosteus platostomus*). The garfish was chosen for previous studies of transverse relaxation in our lab because the garfish can provide both myelinated and nonmyelinated nerves (Fenrich 1992). These nerves are used as simplified models of white matter. The olfactory nerve provides a homogeneous source of long nonmyelinated axons (Easton 1971). On the other hand, the trigeminal and optic nerves are myelinated with Schwann cells and

oligodendrocytes, respectively. Therefore, three unique nerves can readily be obtained from one specimen. The pike, an abundant fish in Alberta, also can provide a nonmyelinated olfactory nerve. Unfortunately, the nerves are not as long and the dissection is more difficult (Easton 1965).

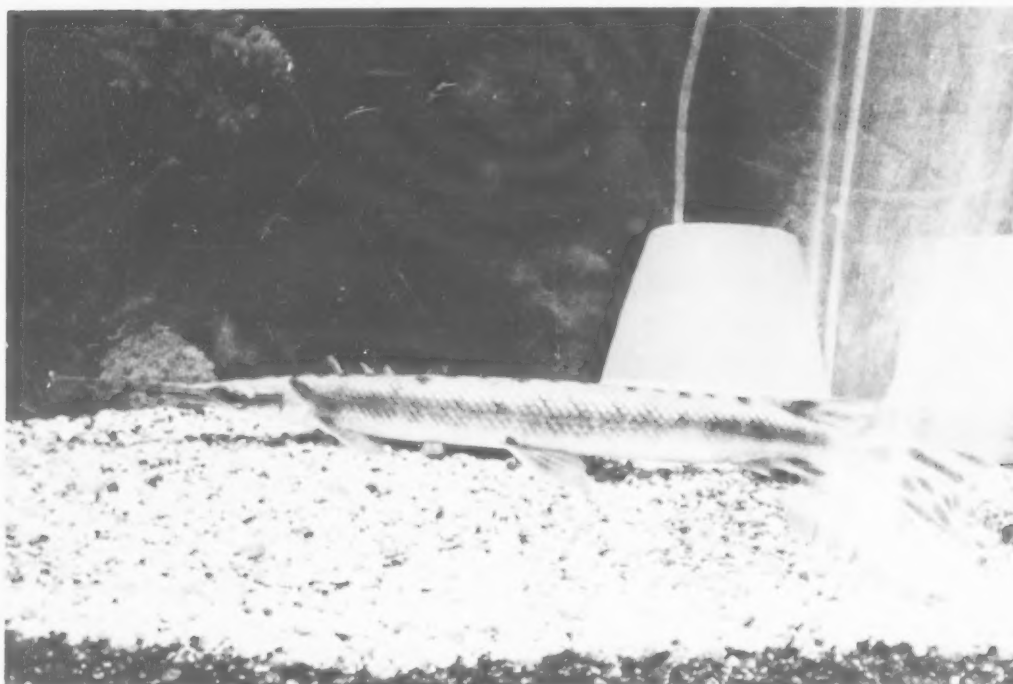


Figure 3.1 Photograph of long-nosed garfish.

The number and type of garfish used in each set of our experiments are presented in Table 3.1. Others have shown that there are no obvious differences in the characteristics of the nerves from these different species (Easton 1965). Fish from the Tropical Fish Gallery (Edmonton, Alberta) were delivered live on the day of the experiment. Fish received from Osage Catfisheries (Osage Beach, MO) were maintained in water tanks in the Health Sciences Lab Animal Building, and their diet consisted of live goldfish. The live garfish were transported in water filled boxes to the NMR lab on the day of the experiment.

Table 3.1 Number and type of garfish that were studied for various experiments that are described in this thesis.

Chapter	Measurement	Spotted Garfish	Long-nosed Garfish	Short-nosed Garfish	Total Garfish
4.1	diffusion	7 ^b	4 ^c	0	11
4.1 ^a	diffusion	0	5 ^c	0	5
4.3	diffusion	2 ^d	4 ^d	0	6
5	diffusion	0	4 ^c	2 ^c	6
7	magnetization transfer	0	0	4 ^c	4

^a Measurements of diffusion in vinblastine-treated nerves.

^b Very young, small garfish (20-30 cm length) obtained from Tropical Fish Gallery, Edmonton.

^c Long, young garfish (40-50 cm length) obtained from Osage Catfisheries, Osage Beach, MO.

^d Larger garfish (40-65 cm length) obtained from Osage Catfisheries.

^e More mature garfish (50-65 cm length, far greater girth) obtained from Osage Catfisheries.

3.1.1.2 Excision of Nerves

The fish were anaesthetized by adding ~ 0.5 g ethyl 3-aminobenzoate methanesulfonic acid salt (also referred to as MS-222) (Aldrich) for every 3.5 L of water. The dosage of MS-222 was increased about three-fold for the very large short-nosed garfish. The fish were decapitated and the lower jaw was removed. The larger fish required the use of a gross anatomy rongeur to cut through their tough scales prior to decapitation. Transverse, shallow cuts were made every 1-2 cm of the rostrum (i.e. snout) using a Dremel tool equipped with a small circular blade. The terminal 0.5 cm of the rostrum containing the olfactory cell bodies was cut off. The remaining pieces of the rostrum were snapped off and pulled carefully from the distal end leaving two olfactory / trigeminal bundles attached to the proximal end. The nerves were doused with buffer throughout the aforementioned procedure to prevent dehydration. The two sets of olfactory and trigeminal nerves could then be cut from the proximal end. The optic nerves were exposed by peeling the bony skull back and removing the fatty tissue in the region behind the eye. The optic nerves were removed by cutting the nerve near the back of the eyeball and near the frontal part of the brain. The nerves

were kept moist during the dissection. The entire dissection procedure took about 25 minutes. Subsequently, with the aid of a dissecting microscope, the olfactory and trigeminal nerves were separated from one another, and blood vessels that normally adhere to the nerves were removed. Also, the outer connective tissue sheath of the optic nerve was removed and discarded. To decrease the degree of handling, the nerves, in most cases, were tied individually with a length of fine thread near one end of the nerve. The nerves could then be moved or manipulated via the thread. The excised nerves were stored in refrigerated buffer (pH titrated to ~ 7.05 using NaOH) containing 125 mM NaCl, 3.5 mM KCl, 3.5 mM CaCl_2 , 24 mM glucose, 10 mM NaHCO_3 , and 3 mM HEPES (Easton 1971) prior to their use in our NMR measurements.

Typical nerve diameters were ~ 0.3 mm, 0.15 mm, and 0.8 mm for the olfactory, trigeminal, and optic nerves, respectively, in the less mature garfish. Also, the length of the olfactory and trigeminal nerves ranged from 2 - 6 cm whereas the optic nerve was only ~ 0.5 cm long. However, the large mature short-nosed garfish had larger nerve diameters of ~ 1 mm, 0.5 mm, and 1 mm and lengths of 6 - 7 cm, 6 - 7 cm, 1 - 1.5 cm for the olfactory, trigeminal, and optic nerves, respectively. Therefore, better signal-to-noise was obtained in our measurements of the nerves from the mature short-nosed garfish.

3.1.1.3 Preparation of Nerve for NMR Measurements

As soon as possible after the dissection, the nerves were prepared for our NMR measurements. Often, several nerves of one type would be pooled together to improve the signal-to-noise of our measurements. The very long olfactory and trigeminal nerves would often be cut in half and the pieces of nerve would be pooled into one sample. In the NMR diffusion measurements presented in this thesis, the excised garfish nerves were immersed in paraffin oil just prior to their NMR measurements and were individually aligned in 1 mm inner diameter capillary tubes. The ends of the tube were sealed with plasticine or teflon tape. This arrangement permitted the measurement of diffusion parallel and perpendicular to the length of the nerve. For the measurements of magnetization transfer (MT), the nerves were immersed in paraffin oil and, since directional dependence was not an issue for MT,

were coiled at the bottom of a 5 mm outer diameter NMR tube. After the NMR measurements, the nerves were re-immersed in buffer. The nerves were not immersed in buffer *during* the NMR measurements because the signal from nerve water would be contaminated by the buffer water. To prevent this signal contamination and also to prevent water loss from the nerve (i.e. dehydration), the excised garfish nerves were immersed in paraffin oil during the NMR measurements. Others have used light oils to prevent dehydration of their samples of tissue (Hodgkin 1956, Bunch 1969). Electron microscopy sections and action potential measurements of several nerves following their NMR evaluation showed no evidence of modification by exposure to paraffin oil on the time scale of our experiment. The times of the NMR measurements after decapitation of the garfish varied since we had three different types of nerve to cycle through the multitude of measurements. Typical ranges of measurement times after dissection were 0.5 - 11 hrs, 1 - 8 hrs, 1 - 16 hrs, and 1.5 - 15 hrs for the measurements presented in Sections 4.1, 4.3, 5, and 7, respectively.

3.1.1.4 Validation of Nerve Integrity and Viability

The viability and structural integrity of the nerves throughout the duration of the NMR experiment was confirmed with periodic electron microscopy (EM) studies and action potential measurements.

Freshly excised nerves were fixed in a cacodylate buffer containing 2.5% glutaraldehyde and post-fixed in buffer containing 1-2% OsO₄. The nerves were then dehydrated in a graded series of ethanol and then embedded in an Araldite CY212 mixture. For EM, transverse and longitudinal ultrathin sections, 60-90 nm in thickness, were stained with uranyl acetate and then lead citrate. The sections were observed on a Hitachi H-7000 transmission electron microscope (Dr. M. Chen, Surgical and Medical Research Institute). The garfish nerves were also fixed at 8 hours after excision to verify the structural integrity of the nerves over the usual experimental time (EM sections not shown). The vinblastine treated nerves (Section 3.1.1.5) and the in-vitro degenerated nerves (Section 3.1.1.6) were fixed in a similar manner to verify the depolymerization of the microtubules and to verify the structural degradation of the nerves, respectively. Representative electron micrographs of transverse and longitudinal sections from the three types of garfish nerve (freshly

excised) demonstrate the structural integrity of the nerves post-excision (Figs. 3.2, 3.3, and 3.4).

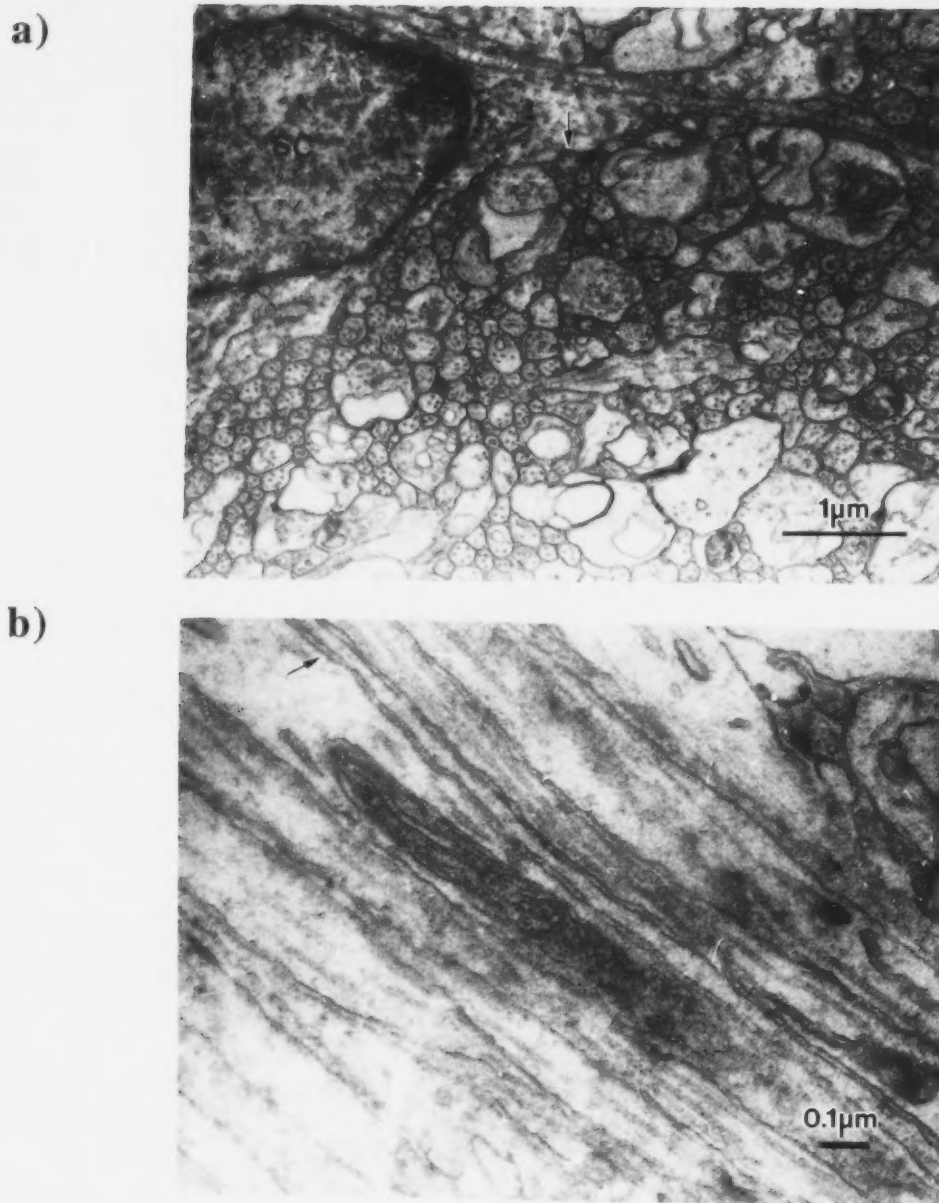


Figure 3.2 Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised olfactory nerve of the garfish. (a) The small ($\sim 0.25 \mu\text{m}$ diameter), circular, relatively homogeneous nonmyelinated axons (arrow) are packed together tightly. Microtubules (seen as small dots within the axons) and a Schwann cell nucleus (SC) are visible. However, several axons appear swollen beyond their normal dimensions. (b) Axonal membranes (arrow) run diagonally across the micrograph.

a)



b)

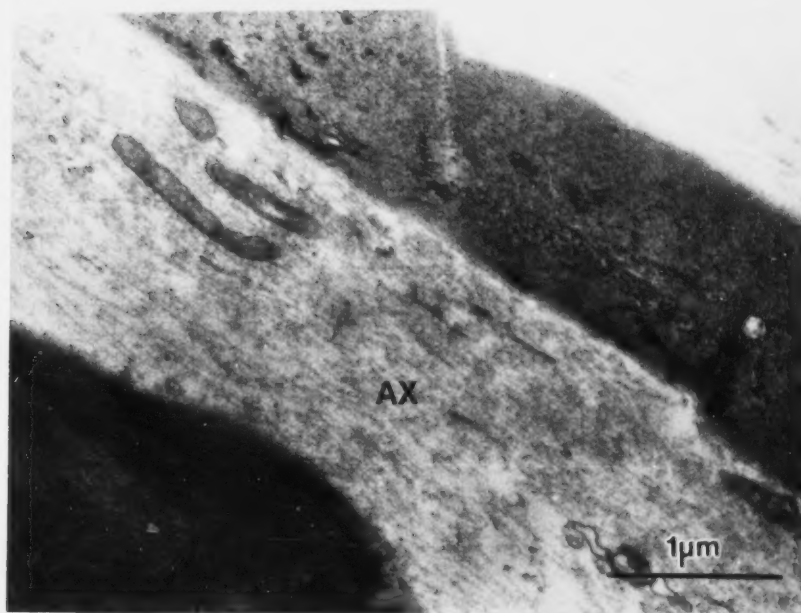


Figure 3.3 Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised trigeminal nerve of the garfish. (a) The axons (AX) have diameters $\sim 3 - 6 \mu\text{m}$ and appear to have good circular shapes which is indicative of a structurally sound nerve. Furthermore, the myelin sheaths (MY) are intact. In peripheral nerves, axons are myelinated by Schwann cells (SC). (b) Numerous asymmetric filaments are visible within an axon (AX).

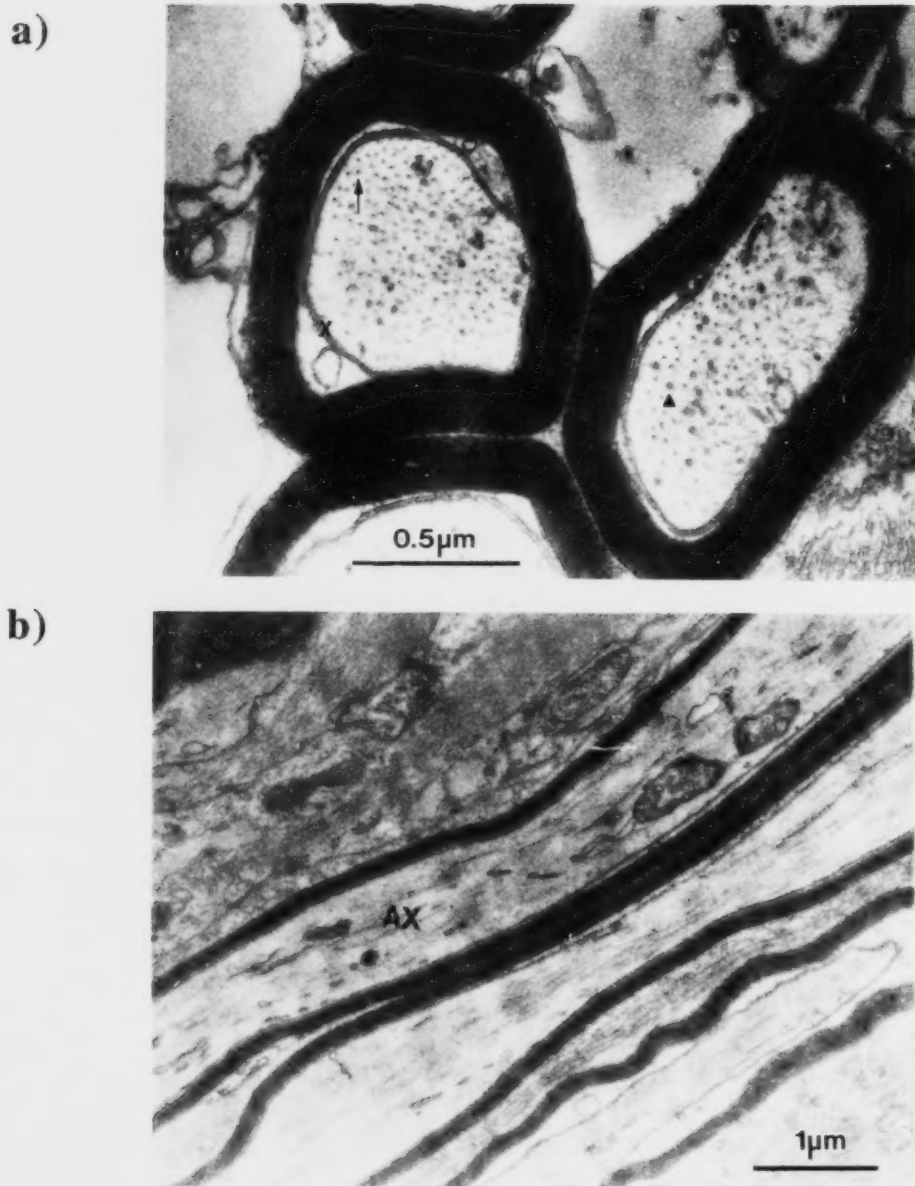


Figure 3.4 Electron micrographs of transverse (a) and longitudinal (b) sections from a freshly excised optic nerve of the garfish. (a) The cytoskeletal elements, namely microtubules (arrowhead) and neurofilaments (arrow), are visible within the intact axons ($\sim 1 \mu\text{m}$ diameter). Microtubules have a donut-shaped cross-section and are $\sim 25 \text{ nm}$ in diameter whereas neurofilaments do not have a hollow central core and are only $\sim 10 \text{ nm}$ in diameter. The multi-lamellar nature of the myelin sheath (MY) is evident. The axonal membrane (x) is also visible. (b) The longitudinal structure of the nerve is evident as three myelinated axons (AX) traverse diagonally across the micrograph.

Action potential measurements of the olfactory and trigeminal nerves from the long-nosed garfish proved the viability of the freshly excised nerves. Action potentials were not measured for the optic nerve because it was too short for the available apparatus. The action potential apparatus was borrowed from Dr. Bill Dryden (Dept. of Pharmacology) and was modified by Narc Ouellette (Dept. of Applied Sciences in Medicine). It consisted of a pulse generator, an electrode system for stimulating the nerves and recording the action potentials, an amplifier, and a digital oscilloscope to view, average, and store the data. The separation between the stimulating and recording electrodes was ~ 1.45 cm. A 2 V, 50 μ s pulse (trigeminal) or 7.5 V, 1 ms pulse (olfactory) with a repetition frequency of ~ 10 pulses/s was determined empirically to yield good action potentials in the nerves. The measurements were performed at room temperature (~ 20 °C).

Action potentials were recorded at $\sim 2, 3, 4, 5.5, 7.5, 11, 23,$ and 30 hours after nerve excision. Between measurements of action potentials, the nerves remained immersed in buffer. Olfactory nerves were capable of transmitting action potentials up to 11 hours post-excision (i.e. no action potentials were observed at 23 and 30 hours). On the other hand, trigeminal nerves showed action potentials throughout the 30 hour time period. Representative action potentials for the olfactory and trigeminal nerves at 2 and 7.5 hours post-excision are presented in Fig. 3.5. There is a noticeable difference between the action potentials of the myelinated trigeminal nerve and the nonmyelinated olfactory nerve. The action potentials peak at ~ 0.6 ms and ~ 60 ms after the initial stimulation pulse for the trigeminal and olfactory nerves, respectively. Given the 1.45 cm distance between the stimulating and recording electrodes, the conduction velocities of the action potentials are ~ 24 m/s and 0.24 m/s for the trigeminal and olfactory nerves, respectively. As expected, the conduction velocity of the nonmyelinated nerve is much (100 times) slower than the myelinated nerve. These values of the conduction velocity are in agreement with values expected for nonmyelinated and myelinated nerves (Easton 1965, Nicholls 1992a). We also observed action potentials in garfish nerves that had previously been immersed in paraffin oil during measurements of diffusion (data not shown).

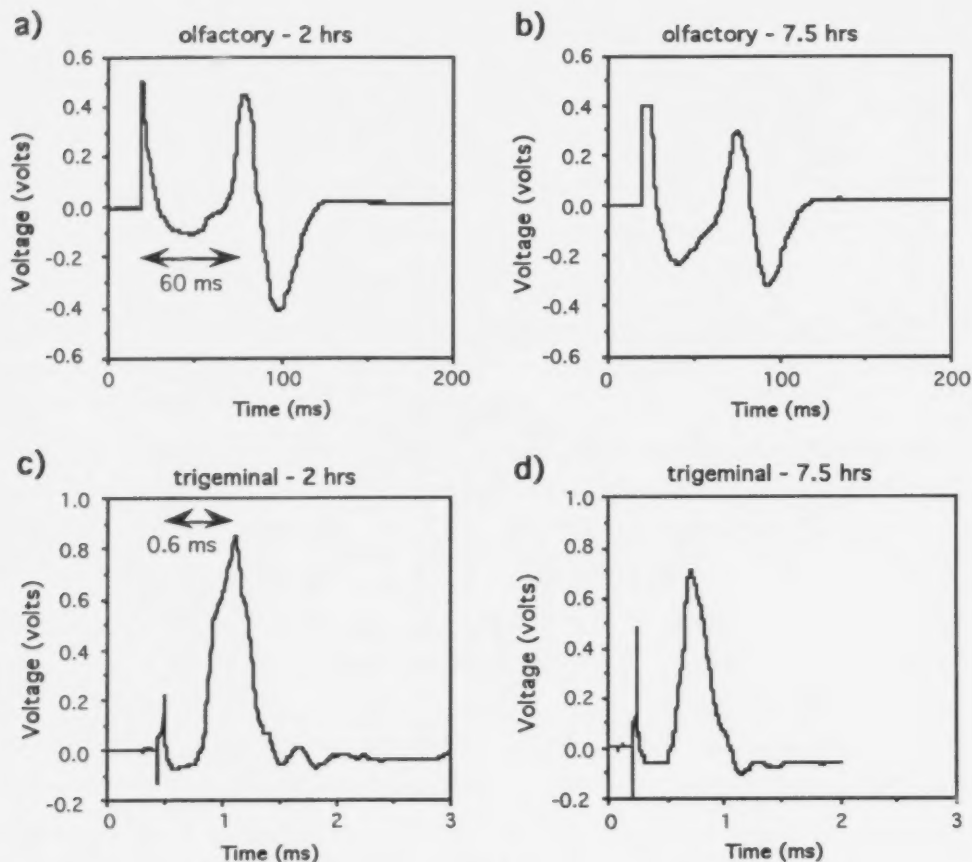


Figure 3.5 Action potentials in the nonmyelinated olfactory (**a, b**) and the myelinated trigeminal (**c, d**) nerves of the garfish at 2 and 7.5 hours post-excision. The time between the stimulating pulse and the first lobe of the action potential is ~ 60 ms (**a, b**) and 0.6 ms (**c, d**) for the olfactory and trigeminal nerves, respectively.

3.1.1.5 Vinblastine Treatment of Garfish Nerves

Because vinblastine is known to depolymerize microtubules and inhibit fast axonal transport (Smith 1975), the intermediate vinblastine treatment was applied to the nerves from 5 long-nosed garfish by immersing them for 48 hours in refrigerated garfish buffer solution containing 0.5 mM vinblastine sulfate (Aldrich). EM sections of these nerves (Figs. 4.2 and 4.3) confirmed the elimination of the microtubules within the axons without visibly affecting the neurofilaments (also see Figs. 1 and 26

in Bensch 1969 for a nice demonstration of the elimination of microtubules upon vinblastine treatment). Additional evidence of microtubular disassembly was the contraction of the olfactory nerve to typically 1/2 to 2/3 of its original length. The trigeminal and optic nerves did not contract so noticeably, likely because the dominance of neurofilaments in their cytoskeleton makes them less dependent on microtubules for their structural integrity.

3.1.1.6 In-Vitro Degenerated Nerves

Four sets of garfish nerves were allowed to degenerate structurally by being maintained in refrigerated buffer for 2 months post-excision, their structural degradation being confirmed with EM sections (not shown). Measurements of diffusion were performed on these degenerated nerves and the results are presented in Section 4.1. We realize that this "model" of in-vitro degeneration is not applicable to in-vivo degeneration. In-vivo, macrophages would be continually removing degenerating debris whereas, in-vitro, the debris accumulates within the nerve. The degenerated olfactory nerve was devoid of intact axons or any regular structure. Various debris was haphazardly distributed throughout the section of "nerve". The degenerated trigeminal and optic nerves showed some interesting features. The myelin decomposed into aggregates of numerous small ovoids that covered the region previously occupied by axons. In addition, the myelin layers were splitting apart in the small amount of persistent myelin. Needless to say, the degenerated nerves were in a state of disarray.

3.1.2 Squid

3.1.2.1 Description and Source

Cephalopods possess the most advanced nervous system of all invertebrates. In 1936, Young discovered that the stellar nerves in the mantle of the squid (Fig. 3.6) possess giant axons with axonal diameters that can measure up to 1000 μm (Young 1936). The large fibers allow fast impulse conduction and, therefore, permit rapid contraction of the mantle which enables the squid to quickly propel itself from danger

(Young 1938). Because of its unusually large size, the giant axon of the squid has provided a workable model of the nervous system for a study of neural transmission (Hodgkin 1939) as well as the structure and properties of axoplasm (Brown 1990). Many of the studies of the squid giant axon are applicable to the understanding of much smaller mammalian axons, such as our own.



Figure 3.6 Photograph of squid.

Loligo pealli is found along the east coast and is the most commonly studied squid. However, it can live for only about 24 hours in captivity and, therefore, must be caught fresh each day. Therefore, studies of the squid giant axon were usually seasonal (May - September) and had to be performed at marine institutes like the famous Marine Biological Laboratory in Woods Hole, MA. This may help to explain the lack of NMR measurements on squid. To the best of my knowledge, the only other NMR study of squid giant axons was the measurement of water proton T_1 's and T_2 's using a portable spectrometer at Woods Hole (Chang 1980). However, 12 years of basic culture research by researchers at the University of Texas Medical Branch at

Galveston has yielded a hardier squid, the *Sepioteuthis lessoniana*, that can be held in captivity for up to 9 months and is capable of being transported live to other labs. The *Sepioteuthis lessoniana* is a viable alternative to *Loligo pealli* since they have the same anatomical features. We purchased *Sepioteuthis lessoniana* from the National Resource Center for Cephalopods, Marine Biomedical Institute, Galveston, TX (Contact: Phil Turk). The squid (one at a time) were shipped live in a sealed plastic bag with oxygenated water. The live squid arrived at our lab ~ 20 hours after departure from their facility. The transport of the squid from Galveston to Edmonton was also an experiment for their facility since they had never previously shipped live *Sepioteuthis lessoniana* over such a large distance. To our good fortune, their experiment was a success.

3.1.2.2 Excision of Nerves

The live squid had an average mantle length of 16 cm. The hindmost stellar nerves (and often their adjacent stellar nerves) were excised from five squid, and cleaned of connective tissue and smaller axons to isolate the giant axons (Fig. 4.5). I used a procedure that was taught to me by Joe DeGiorgis, a graduate student in Tom Reese's lab (based at NIH), at the Marine Biological Laboratory in Woods Hole, MA. The dissection procedure is described in more detail in the literature (Adelman 1990). First, a dissection table for squid had to be constructed. It contains a small well in which the squid mantle can be worked on while the mantle is bathed in cold artificial sea water (ASW). The bottom of the well is made of glass. A bright light (from an x-ray box for example) is placed underneath the glass window. This allows the illumination of the mantle and hence, visualization of the nerve fibers in the mantle (i.e. the "*calamari*" part of the squid). The well was continually perfused with fresh ASW.

The squid must be live and undamaged so that the muscle of the mantle is transparent and the nerves can then be visualized through the mantle. Once the squid is dead, the following dissection procedure must be performed quickly and precisely because visualization of the nerves in the mantle becomes progressively poorer (and then impossible) as the mantle becomes opaque. After decapitation, the mantle was cut along the midline {side opposite of the pen (i.e. cartilage)}, and the mantle cone was

opened to form a triangle. The mantle is placed immediately into the cold ASW in the well. The digestive gland, gills, ink sac, pen, etc. were removed carefully from the mantle. The skin was removed from the exterior of the mantle. Subsequently, the cleaned mantle was pinned to the bottom of the well and several stellar nerves can be seen emanating from two ganglia (one on each half) near the base of the mantle. The following description is for the dissection of the hindmost stellar nerve (i.e. closest to the midline), but the procedure is similar for the adjacent stellar nerves. At the bifurcation of the nerve, fine scissors were used to cut a small shallow groove through the mantle along the top edge of the stellar nerve. A suture thread was passed underneath the nerve with forceps and the nerve was ligated in order to prevent any loss of axoplasm. The nerve was cut distal to the tie. The nerve can be pulled out carefully with the thread (may have to use scissors to aid in opening the groove) until the ganglion is reached. The nerve can be tied near the ganglion and then cut between the ganglion and the tie. All four stellar nerves closest to the midline (two a side) were removed from the mantle (sometimes only two or three were removed successfully) and stored in cold fresh artificial sea water buffer (pH titrated to ~ 7.4 using NaOH) containing 430 mM NaCl, 10 mM CaCl_2 , 50 mM MgCl_2 , 10 mM KCl, and 10 mM HEPES (Adelman 1990). This dissection procedure took about an hour. Subsequently, with the aid of a dissection microscope, the giant axons were isolated from the stellar nerves by peeling away the connective tissue and smaller axons. One must be careful in order not to damage the axonal membrane. In this procedure, the nerve is held outstretched (must be taut) at the bottom of a petri dish (bottom covered with a silicone-type gel) by inserting two small pins through the thread at either end of the nerve. The giant axon can be identified by its translucent appearance. The cleaning of the axons took ~ 30 minutes per nerve. The mean giant axonal diameter was $300 \pm 80 \mu\text{m}$, and typical lengths were 3 cm. The giant axons were stored in ASW buffer prior to our NMR measurements of diffusion. To prevent contamination of the NMR diffusion measurements by water signal from the buffer, the externally dried axon was immediately placed in paraffin oil to prevent water evaporation and was aligned in a 1 mm inner diameter capillary tube (as in the garfish studies). NMR measurements of diffusion were performed on 15 giant axons that were obtained from 5 squid.

3.1.2.3 Validation of Nerve Integrity

Upon visible inspection, most of the axons appeared quite clear and relatively free of white spots (which would indicate damage if present) or stretched regions. The structural integrity of the freshly excised axons was confirmed with both light and electron microscopy (Fig. 4.5). The axons were fixed in a solution of 2.5% glutaraldehyde in artificial sea water buffer. The fixed nerves were prepared for LM and EM using routine methods (see Section 3.1.1.4). However, LM utilized thick sections whereas the EM utilized ultrathin sections. It has been demonstrated that dissected squid giant axons maintained in buffer (sea water) could conduct action potentials for ~ 12 hours or more (Keynes 1958). Our diffusion measurements on the giant axons were performed within the range of 1.5 to 6 hrs (mean time $\pm$ standard deviation = 3.5 ± 1.5 hrs) after squid sacrifice.

3.1.3 Frog

3.1.3.1 Description of Model of Wallerian Degeneration

A convenient and universally accepted model for studying nerve degeneration in-vivo is the portion of peripheral nerve that is distal to an inflicted nerve injury. For example, crushing a peripheral nerve, which interrupts axonal continuity with the cell body, causes Wallerian degeneration to occur distal to the crush. Wallerian degeneration is characterized by structural changes such as axonal dissolution, followed by contraction and fragmentation of myelin, and finally disintegration and removal of myelin and other debris by Schwann cells and macrophages (Schroder 1975). Depending on the temperature and species of interest, this degenerative process can occur at different rates. The peripheral nerve we chose to study is the sciatic nerve of the frog (*Xenopus laevis*). The sciatic nerve of the frog is easily accessible and the frogs are relatively inexpensive. Furthermore, there was considerable experience with this particular model within our department. The downside of this animal model is that Wallerian degeneration is normally delayed in onset and proceeds more slowly in cold-blooded animals (such as amphibians) than in mammals (Sunderland 1968). Diffusion measurements of water in uninjured and Wallerian degenerated peripheral nerve are presented in Chapter 6. In a separate

study, various diffusion measurements of uninjured frog nerve were performed and are presented in Section 4.3.

3.1.3.2 Nerve Preparations

Female specimens weighing 125-150 g were housed in water-filled tanks at room-temperature and fed frog brittle (Nasco, Fort Atkinson, WI). Following anaesthesia with a 2% aqueous solution of urethane, surgery was performed on animals. For six animals, the sciatic nerve in one leg was exposed at the upper-thigh level and crushed with a nylon thread drawn tight against a glass rod for 30 seconds, thereby creating an injury that allowed regeneration of nerve fibers to occur. The crush procedure has been shown histologically to interrupt all nerve fibers and to allow regeneration to occur (Chan 1989). In a second group of five animals, the nerve from one leg was tied at two sites separated by an interval of 2-4 mm, the ties remaining attached to the nerve for the duration of the study so as to prevent the regeneration of fibers. The skin incision was closed following all procedures and the animals, after recovery, were returned to their holding tanks. Control measurements were obtained from the contralateral uninjured nerve of all of the above frogs, plus the nerves of four additional normal frogs (two nerves each). These additional uninjured nerves were also used for the measurements that are presented in Section 4.3.

Four weeks following surgery the animals were again anaesthetized and, following decapitation, both the injured and uninjured (normal) nerves were removed and placed in frog buffer (112 mM NaCl, 3 mM KCl, 3 mM CaCl₂, 1.6 mM MgSO₄, 3 mM HEPES, and 5.5 mM glucose; pH = 7.4) for short term storage immediately prior to the diffusion measurements. The degenerated sciatic nerves were excised at four weeks post-surgery because the nerves have been shown to be sufficiently degenerated by this time (Does 1995a). The exterior connective tissue sheath was not removed from the nerves. The lengths of excised nerve ranged from 1.0 to 2.5 cm, the length beginning immediately distal to the crush or tie in the case of injured nerves. The diameter of the nerves was ~ 1 mm. To prevent errors in the NMR diffusion measurements caused by water signal contamination from the buffer and also to prevent water evaporation, each of the nerves was placed in paraffin oil and aligned individually in a 1 or 1.5 mm inner diameter capillary tube immediately prior to the

NMR experiment. Diffusion measurements on the freshly excised sciatic nerves (without buffer) were performed within 15 to 20 minutes of nerve excision.

3.1.3.3 Validation of Integrity/Degeneration in Uninjured/Injured Nerves

Immediately following the diffusion measurements, the nerves were fixed for light (LM) and electron microscopy (EM) as described in the literature (Chan 1989). Micrographs were obtained to determine the normal and the injured conditions of the nerves (see Figs. 6.1 - 6.3). LM and EM sections taken at one-third and two-thirds along the length of the excised nerve indicated that the damaged nerves had undergone relatively uniform degeneration. The LM and EM observations of the degenerated sciatic nerves are in agreement with other descriptions of nerve during Wallerian degeneration (Nathaniel 1963, Schroder 1975). The degenerated nerves were larger in diameter, yielded greater NMR signal, and were more grayish in appearance than the uninjured nerves. In addition, the frogs, which had the crushed or tied sciatic nerve, had difficulty swimming. These observations gave us confidence in our model of nerve degeneration.

3.2 Methodology for Measurements of Diffusion

The hardware that was used for the measurements of diffusion are described in Section 3.2.1. In addition, the NMR pulse sequences and their parameters are discussed in Section 3.2.2. Our various methods of measuring diffusion were validated on samples with known diffusion coefficients, such as pure water. These diffusion techniques were used for the bulk of the measurements that are presented in this thesis (Chapters 4, 5, and 6).

3.2.1 Hardware

All of our measurements were performed on an in-house modified 40 cm horizontal-bore, 2.35 T Bruker CXP spectrometer (1984 vintage model) with an Aspect 3000 computer system, Adakos operating system, and Tomikonn software. The radiofrequency (rf) amplifiers that were used during the course of this work were

from Amplifier Research (AR) (2 kW) and Advanced Microwave Technologies (AMT) (1 kW). The Bruker gradient drivers could generate a maximum of ~ 30 A and had a rise/fall time of ~ 1 ms. The pre-emphasis unit was not employed. A VAX 11/750 and a DEC station 3200 were used for most of the data analysis. The gradient and rf coils are described in the next sections.

3.2.1.1 Gradient Coils

Gradient coils were designed specifically for our measurements of diffusion in excised samples of nerve. The impetus for designing these new gradients was our difficulty in making reproducible and accurate measurements of diffusion in known samples using a home-made gradient set (maximum gradient strength ~ 12 G cm $^{-1}$) that was available at the start of this project. The two primary problems were eddy currents and poor reproducibility of the gradient pulses (i.e. $G_1 \neq G_2$). The gradient strength (G) or length of the second gradient pulse had to be trimmed (i.e. increased or decreased) in order to achieve an echo with maximum amplitude at the proper timing, and therefore, to measure correct diffusion coefficients. The amount of trimming was dependent on the spacing between the pulses, the length of the pulses, and their gradient strengths. To avoid this problem and since we needed larger gradient strengths, a new set of gradient coils was designed and built by our group. Karim Damji (Electrical Engineer, NMR Group) designed the new gradient coils, in conjunction with Dan Doran (Technician, NMR Group) and myself. The actual physical construction of the gradient coils was performed by Dan Doran and myself.

The prerequisite properties of the gradient coils were as follows. We needed a region of linearity of ~ 2 cm (± 1 cm about the origin). This was offset by the desire to have the strongest possible gradient strength. Strong gradients have the advantage of allowing the use of shorter gradient pulse lengths and diffusion times, and still achieving sufficient echo attenuation to measure accurately the diffusion coefficient. It is desirable to have fast rise/fall times, and therefore, we had to make sure that the rise/fall times of the gradients were not much slower than the rise/fall time of the gradient drivers. In order to improve the ability of the gradients to yield reproducible and stable gradient pulses, the whole assembly, including the wires, had to be extremely stable and rigid to not allow any motion. We only required two directions

(instead of the usual three) for the gradients since we were interested in measuring diffusion in solely two orthogonal directions with respect to the long axis of the nerves (i.e. parallel and perpendicular).

A Maxwell pair was used to generate a magnetic field gradient in the z direction, G_z , (parallel to B_0) whereas a "flattened Golay" was used to generate a gradient in the x direction, G_x (perpendicular to B_0) (Fig. 3.7) (Mansfield 1982a). The static magnetic field is along the z direction, and therefore, $G_z = \partial B_z / \partial z$ and $G_x = \partial B_z / \partial x$. Assuming a maximum current of 30 A, and 25 turns of wire for G_z and 36 turns of wire for G_x , analytical calculations estimated maximum gradient strengths of 151 G cm^{-1} and 58 G cm^{-1} for G_z and G_x , respectively. Numerical simulations also confirmed the expected maximum gradient strengths ($G_z \sim 144 \text{ G cm}^{-1}$ and $G_x \sim 68 \text{ G cm}^{-1}$) and that the gradients, as designed, should be linear over the desired $\pm 1 \text{ cm}$ (data not shown). Assuming a maximum resistance of 0.8Ω , further calculations by K. Damji predicted rise/fall times of $145 \mu\text{s}$ and 1.4 ms for the z and x gradient coils, respectively.

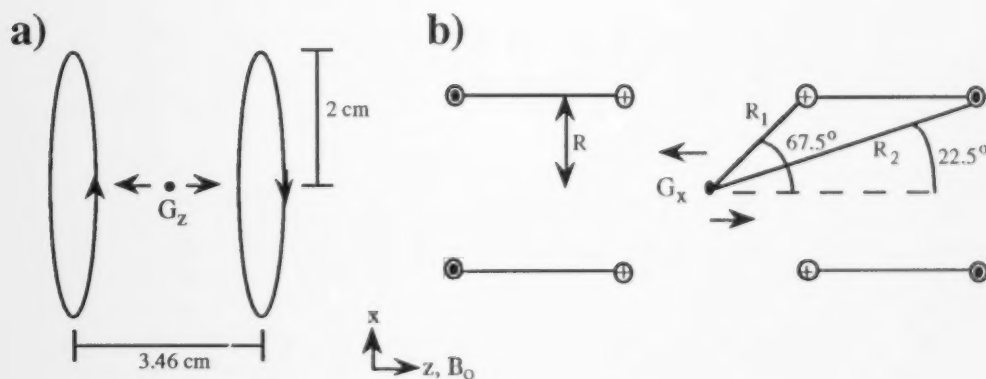


Figure 3.7 Wire arrangements and current directions that were used to generate a magnetic field gradient parallel (a) and perpendicular (b) to the static magnetic field, B_0 . (a) Maxwell pair. (b) Flattened Golay with $R = 3.25 \text{ cm}$, $R_1 = 3.52 \text{ cm}$, and $R_2 = 8.49 \text{ cm}$ (diagrams not to scale). The magnetic field, B_z , adds or subtracts to B_0 in a linear fashion around the origin.

Appendix 1 provides schematic diagrams of the gradient coils and a brief description of their construction. G_z was calibrated by the "FID zero" method of Murday (Murday 1973). Our cylindrical sample was pure water in an NMR tube (0.42 mm inner diameter) and this sample was oriented perpendicular to G_z . We used a spin-echo sequence with gradient pulses in the first (G_1) and second (G_2) intervals. The gradient strengths had ratios of 3 to 1. Thus, the duration of G_2 had to be 3 times the duration of G_1 to center the echo maximum at the proper echo time. The echo was acquired during the central portion of G_2 . The strength of G_2 was kept quite weak ($< 4 \text{ G cm}^{-1}$). The timings between the zero-crossings of the echo were measured and G_z was calculated from Eqn. 2.51. G_x was calibrated by measuring the echoes from a PGSE diffusion measurement of pure water and working backwards from the known diffusion coefficient to calculate G_x (as discussed in Section 2.1.5.3). G_z and G_x were calculated to be 89.9 and 35.47 mG/cm/IOD, respectively. The IOD is a parameter of the Bruker spectrometer that is directly proportional to the current that is applied to the gradient coils (i.e. a measurement by a current probe yielded 16.5 mA/IOD). Since the IOD ranges from 0 to a maximum of 1792 (corresponds to a current of 29.6 A), the maximum available gradient strengths were 161 and 64 G cm^{-1} for G_z and G_x , respectively. These experimentally derived gradient strengths are similar to the values that were predicted by the analytical and numerical calculations. The measured resistance, inductance, and intrinsic rise/fall times were 0.635 Ω , 90 - 94 μH , and 142 - 148 μs for G_z and 0.754 Ω , 0.9 - 1.54 mH, and 1.2 - 2.0 ms for G_x . The linearity of the gradients over $\pm 1 \text{ cm}$ was verified by a bench test. The bench test consisted of measuring the voltage induced in a small receiving coil that was placed at various positions with respect to the center of the gradient coils as a pulse of current was passed through the gradient coils.

This set of gradient coils was used for the majority of the diffusion measurements that are presented in this thesis. However, we should note that the measurements on the 11 normal garfish nerves in Section 4.1 were performed using a prototype of the above gradient set. This prototype only had a z gradient and its maximum possible gradient strength was 112 G cm^{-1} (62.4 mG/cm/IOD). Therefore, in this case, measurements of diffusion parallel and perpendicular to the nerves required the use of both saddle and solenoid rf coils, respectively.

3.2.1.2 Radiofrequency Coils

Two types of rf coils were used in our studies. A 5 mm diameter, 1.5 cm long, 6 turn solenoid was used for the majority of our studies. The solenoid was made of silver-coated copper wire. However, a 5 mm diameter, 2 cm long saddle coil was used for some of the measurements that are presented in Sections 4.1 and 4.3, namely the measurements of diffusion when the nerves were oriented parallel to B_0 . The saddle coil was made of thin copper foil that had been etched into the proper configuration (Mansfield 1982b). The rf coils were wrapped around 5 mm outer diameter NMR tubes and glued securely in place with Crazy Glue. Cylindrical formers (made of Teflon so it contains no protons) were designed and constructed to hold the rf coil assembly firmly in place and to center the rf coil (and therefore the sample) reproducibly in the center of the gradient set. These formers also ensure that our asymmetric samples will be aligned parallel or perpendicular to the gradients. The two Teflon formers (with rf coils) were inserted individually into the 3 cm horizontal bore of the gradient set and taped firmly to prevent any vibration. In order to minimize the amount of metal near the gradient coils and to reduce the potential for eddy currents, the match-tune box was placed remotely from the gradient set and was connected to the rf coils with a long length of well secured coaxial cable. The coaxial cable substantially decreased the quality factor (Q) of the rf coils to ~ 50 .

3.2.2 Pulse Sequences and Parameters Used to Measure Diffusion

This portion is subdivided into three parts. The basic pulsed-gradient spin-echo method (3.2.2.1), the bipolar gradient pulse sequence (3.2.2.2), and the CPMG-PGSE (3.2.2.3) methods are presented individually. Elements that are common to all three sequences are discussed in the first portion. All diffusion measurements were performed at room temperature (20 ± 2 °C). The temperature within the magnet was monitored by measuring the temperature of a vial of water that had been in the magnet during the experiment. In all cases, the nerves were immersed in paraffin oil during the diffusion measurements, as discussed in Section 3.1.1.3.

3.2.2.1 Basic Pulsed-Gradient Spin-Echo Pulse Sequence

The following description deals with our routinely used measurement techniques that are pertinent to Sections 4.1, 4.2, portions of 4.3, and 6. The standard Stejskal-Tanner pulsed-gradient spin-echo diffusion sequence (PGSE), modified with 1331 ($\pi/2$) and 2662 (π) binomial rf pulses (Hore 1983), was used to measure the diffusion coefficients (Fig 3.8). The binomial rf pulses were utilized in order to excite the water protons in the nerves selectively and to exclude the proton signal from the bathing paraffin oil (light mineral oil, Fisher Scientific). The 1331 $\pi/2$ and 2662 π binomial rf component lengths were multiples of 1.25 μ s. Using a binomial pulse component separation of 1.316 ms [i.e. $1/(2 \times 380 \text{ Hz})$], the binomial pulses gave rise to an excitation null at 380 Hz from the on resonance water peak at 100.19 MHz, which effectively reduced the paraffin oil signal by about 2 orders of magnitude (Fig. 3.9). The intensity of the water signal was reduced by about 10% when using binomial pulses.

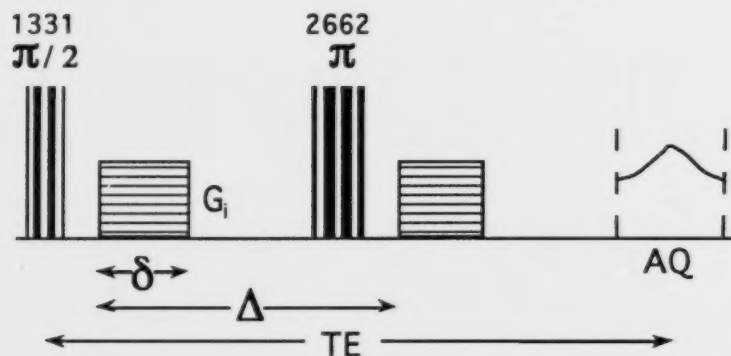


Figure 3.8 Stejskal-Tanner spin-echo pulse sequence modified with 1331 and 2662 binomial rf pulses. The incremented diffusion-sensitizing rectangular gradient pulse is labeled G_i , while δ , Δ , and TE represent constant time intervals. The acquisition time (AQ) for the echo peak was 10 ms.

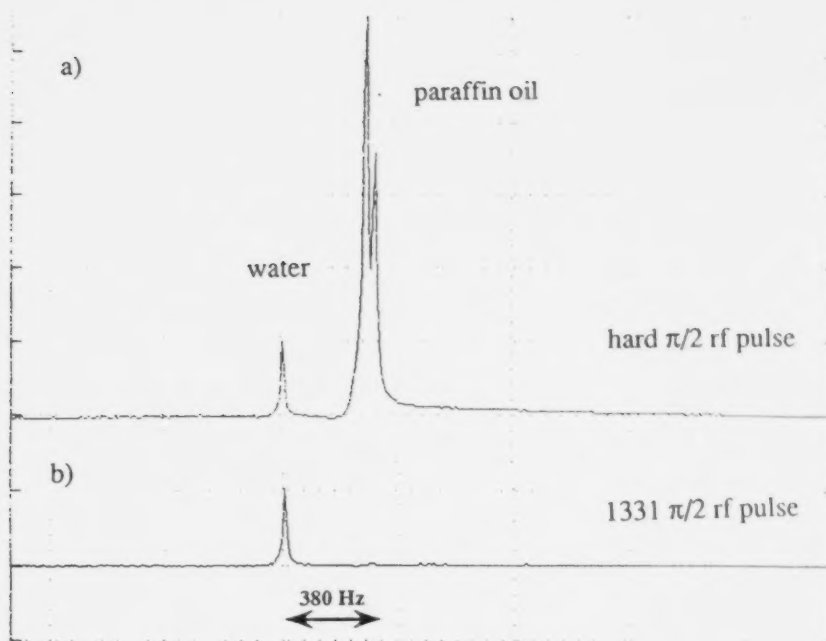


Figure 3.9 Proton spectrum acquired by a hard $\pi/2$ rf pulse (a) and by a 1331 $\pi/2$ rf pulse (b) of a trigeminal garfish nerve that is immersed in paraffin oil. The attenuation of the paraffin oil with preservation of the nerve water signal is evident in (b). Typical nerve water proton linewidths at half-height were 10-20 Hz.

The timings of the diffusion-sensitizing rectangular gradient pulses ($\delta = 6$ ms, $\Delta = 30.128$ ms) were kept constant while the gradient strength (G_i) was incremented to a maximum of ~ 12 G cm $^{-1}$ to yield a maximum gradient factor, $b_{\max}$, of $\sim 1 \times 10^5$ s cm $^{-2}$. The time between the end of the first gradient pulse and the π pulse was set at 20 ms to avoid any spurious eddy currents that may be generated by the gradient pulse. The gradient factors, b , are calculated from Eqn. 2.46. A $b_{\max}$ of $\sim 1 \times 10^5$ s cm $^{-2}$ is typical for diffusion measurements on whole-body clinical magnetic resonance imagers. The spin-echo time (TE) was kept constant at 60 ms. Trimming of the second gradient pulse was not required for the newly constructed gradient set (Section 3.2.1.1). In some instances, the gradient strength was increased to a maximum of ~ 108 G cm $^{-1}$ to yield a $b_{\max} \sim 8 \times 10^6$ s cm $^{-2}$ which allowed measurement of the full diffusion curve. Five and 33 data points were obtained typically for the diffusion curves from $b = 0$ to $b_{\max} \sim 1 \times 10^5$ s cm $^{-2}$ and 8×10^6 s cm $^{-2}$, respectively. D_{NMR}

values were measured parallel ($//$) and perpendicular ($\perp$) to the sample (that is oriented transverse to B_0) by using the x and z gradients, respectively.

Averaged spin-echoes were obtained over 16 scans (4 scans for Chapter 6 measurements) with a repetition interval of 5 s (20 s for distilled water samples). The echo maximum was further averaged over ± 20 of the 1024 echo data points acquired with a dwell time of 9.8 μ s. The echoes (real and imaginary) were magnitude corrected to avoid phase instabilities. To eliminate any D.C. offset, the amplitudes were baseline corrected by subtracting the amplitude of a fully dephased echo. A representative set of echo maxima as the gradient strength was incremented in a PGSE sequence is shown in Fig. 3.10.

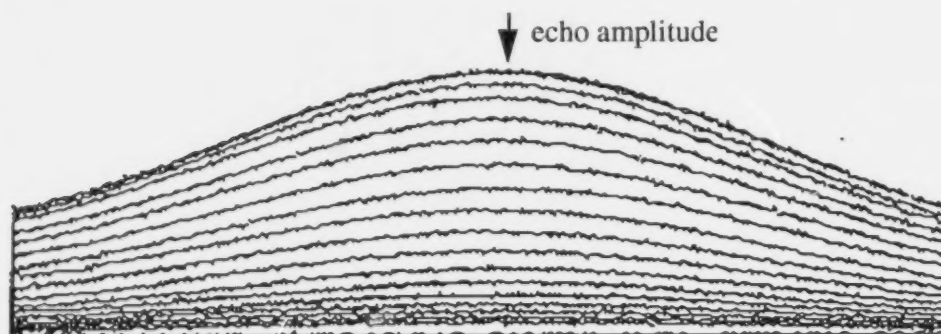


Figure 3.10 Typical echo maxima obtained from a PGSE diffusion experiment. The top echo corresponds to zero applied diffusion-sensitizing gradients. The amplitude of the echoes decrease as the gradient strength is increased. The bottom "echo" corresponds to a signal that is fully dephased by setting $G_1 \neq G_2$. The acquisition window is 10 ms.

D_{NMR} values were obtained from the slope of single-component fits (5 data points per D_{NMR} typically) of $\ln\{\text{echo amplitude } (G_i \neq 0) / \text{echo amplitude } (G_i = 0)\}$ versus b for those diffusion curves with a b_{max} of $\sim 1 \times 10^5 \text{ s cm}^{-2}$. For example, at a b value of $\sim 1 \times 10^5 \text{ s cm}^{-2}$, the amplitude of the echo usually decreases to $\sim 70\text{--}85\%$ and $\sim 20\text{--}40\%$ of the original echo amplitude (i.e. at $b = 0$) for diffusion perpendicular and parallel to the nerve axis, respectively. Therefore, the diffusion coefficients could be measured accurately since there was sufficient signal attenuation.

To verify the linearity of the diffusion curves for single component systems, together with both the accuracy and isotropy of D_{NMR} over the entire range of b , the diffusion curves of pure water and paraffin oil (light mineral oil, Fisher Scientific) phantoms were measured. The diffusion curves are linear for the pure water proton signal (Fig. 3.11) over two orders of magnitude (r^2 of best fit = 1.000) out to $b \sim 2 \times 10^5 \text{ s cm}^{-2}$ and for the paraffin oil proton signal over approximately one order of magnitude (r^2 of best fit = 0.999) out to $b \sim 8 \times 10^6 \text{ s cm}^{-2}$ (Fig. 4.1a). The diffusion coefficients (Table 3.2) were found to be isotropic to within experimental error, and for pure water D_{NMR} was routinely within 3% of published values (Mills 1973). Measurements of diffusion in distilled water were performed often to verify that our experimental setup was still working properly.

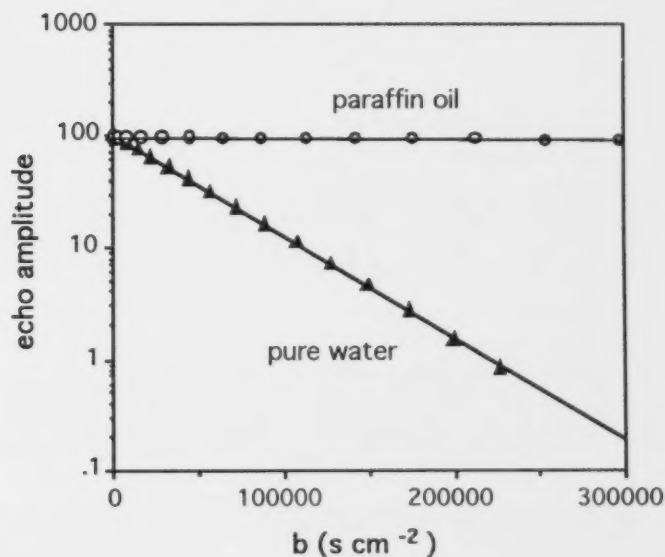


Figure 3.11 Diffusion curves of pure water and paraffin oil. The faster diffusing water loses signal more rapidly than the viscous paraffin oil.

Table 3.2 NMR measurements of diffusion coefficients of pure water, paraffin oil, and water in celery (parallel (//) and perpendicular ($\perp$) to the length of the sample).

Sample	$D_{\text{NMR}}(\text{//})$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\text{//})}{D_{\text{NMR}}(\perp)}$
Pure water at $(22.0 \pm 0.5)^\circ\text{C}$ ^a	2.11 ± 0.06	2.09 ± 0.06	1.0 ± 0.1
Paraffin oil ^{b, c}	0.021 ± 0.002	0.020 ± 0.002	1.0 ± 0.1
Celery ^c			
parenchyma	1.61 ± 0.04	1.65 ± 0.04	1.0 ± 0.1
vascular bundles	1.47 ± 0.09	0.64 ± 0.07	2.3 ± 0.4

^a For a single measurement the error in the best fit was typically $\leq \pm 0.01 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. For repeated measurements the spread in D_{NMR} values was typically within 3% of the expected literature values (Mills 1973), in this case, $2.10 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$.

^b Averaged over five measurements, the errors reflecting the 95% confidence interval.

^c Measurements made at room temperature which varied between 20 and 25 $^\circ\text{C}$.

The ability to measure anisotropic water diffusion was tested on isolated vascular bundles from celery (*Apium graveolens*) petioles prior to the measurements on the samples of nerve. Diffusion measurements on celery petioles showed distinct differences between the parenchyma ("bulk") and the vascular bundles ("strings") (Table 3.2). In agreement with others (Moseley 1991a, Sotak 1992), anisotropy was observed in the vascular bundles (Fig. 3.12) whereas isotropy was observed in the parenchyma. This is not surprising because the cells in the parenchyma are generally isodiametric and hence impart equal diffusion barriers in all directions, whereas those in the vascular bundles are elongated and longitudinally-oriented (Esau 1936).

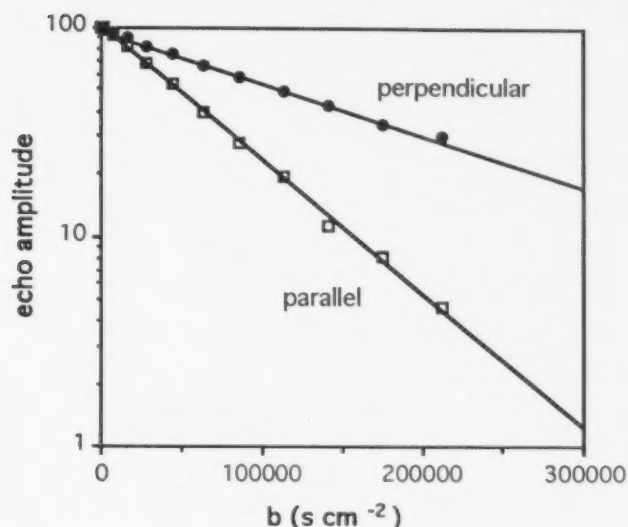


Figure 3.12 Diffusion curves of water in vascular bundles of celery measured parallel and perpendicular to their long axis. Faster diffusion parallel to the bundle causes greater attenuation of the echo.

3.2.2.2 Bipolar Gradient Pulse Sequence

Local magnetic-susceptibility gradients (also called background gradients) can be induced when a heterogeneous sample is placed into a static magnetic field, B_0 . The following description deals with measurement techniques that we used to eliminate the effects of background gradients on the measured diffusion coefficient (pertinent to Section 4.3) Bipolar gradient pulse pairs which are *antisymmetrically* polarized about the π rf pulse in a spin-echo sequence (BGP) can eliminate deleterious cross-term effects on D_{NMR} (Hong 1992). In contrast, the effects of the background gradients on D_{NMR} are not eliminated by bipolar gradient pulse pairs in which the second pair is reversed (BGPR), i.e. which are *symmetrically* polarized about the π rf pulse in a spin-echo sequence. Since the BGP and BGPR pulse sequences can utilize the same timings, the effects of any background gradients can be isolated from the effects of restriction on D_{NMR} . In addition, the BGP and BGPR measurements were compared to PGSE measurements. Three spin-echo diffusion measuring sequences with differing patterns of diffusion-sensitizing gradients (PGSE, BGP, BGPR as shown in Fig. 3.13) were used to measure D_{NMR} . In addition, the orientation of the long axis of

the samples was aligned either longitudinal or transverse to the direction of B_0 for minimum or maximum generation of background gradients, respectively.

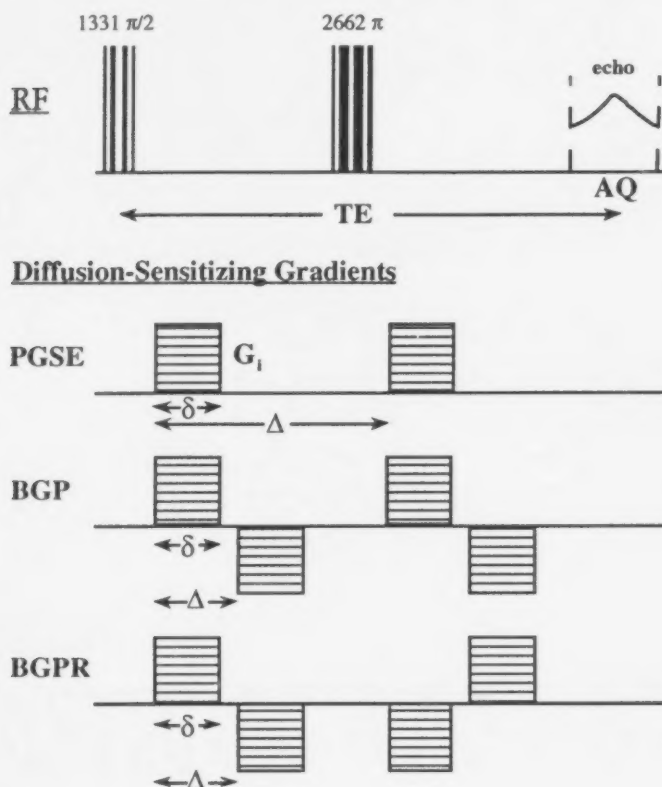


Figure 3.13 Spin-echo diffusion pulse sequence, modified with $1331 \pi/2$ and 2662π binomial rf pulses, with three different patterns of diffusion sensitizing gradients. The acquisition interval (AQ) is 10 ms.

Binomial rf pulses, $1331 (\pi/2)$ and $2662 (\pi)$, were used to excite the nerve water protons selectively (and not the bathing paraffin oil). The binomial rf component lengths were multiples of $1.25 \mu\text{s}$ with binomial component separations of 1.316 ms . The timings of the diffusion-sensitizing rectangular gradient pulses ($\delta = 6 \text{ ms}$, $\Delta = 30.128 \text{ ms}$), where δ and Δ are defined in Fig. 3.13, were kept constant while the gradient strength (G_i) was incremented to a maximum of $\sim 12 \text{ G cm}^{-1}$ and $\sim 9 \text{ G cm}^{-1}$ for the PGSE and the BGP/BGPR pulse sequences, respectively. The

diffusion time, t_d , where $t_d = (\Delta - \delta/3)$, was kept constant at 28 ms for the PGSE, BGP, and BGPR sequences in order to keep the effects of restrictive influences the same. Maximum gradient factors, $b_{\max}$, of $\sim 1 \times 10^5 \text{ s cm}^{-2}$ were typical, where $b = \gamma^2 G_i^2 \delta^2 (\Delta - \delta/3)$ for the PGSE sequence and $b = 2\gamma^2 G_i^2 \delta^2 (\Delta - \delta/3)$ for the BGP/BGPR sequences and where γ is the magnetogyric ratio of the protons. The spin-echo time (TE) was kept constant at 60 ms and 120 ms for the PGSE and BGP/BGPR pulse sequences, respectively. Diffusion coefficients in garfish nerve are shown in Chapter 5 to be independent of the spin-echo time over the range of 60 - 120 ms. Spin-echoes were averaged over 4, 8, or 16 scans, depending on the available signal to noise ratio, with a repetition interval of 5 s (20 s for the distilled water and apple samples). D_{NMR} values were obtained from the slope of a single-component fit (5 data points per D_{NMR}) to a semi-log plot of $\ln\{\text{echo amplitude } (G_i \neq 0) / \text{echo amplitude } (G_i = 0)\}$ versus b .

To verify the accuracy and isotropy of D_{NMR} measurements, diffusion in distilled water was evaluated (see Table 4.6) prior to the diffusion measurements on apple and nerve. As expected, the diffusion in distilled water was isotropic $\{D_{\text{NMR}}(\parallel) = (2.01 \pm 0.04) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $D_{\text{NMR}}(\perp) = (2.01 \pm 0.01) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}\}$ and was within 4% of the expected diffusion coefficient of pure water ($1.94 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) at 19 °C (Mills 1973). Apples are known to possess significant background gradients due to differences in magnetic susceptibility between their constituent air bubbles and the apple flesh (Hong 1992, Lian 1994). To verify consistency with the data of Lian et al. (1994) and Hong and Dixon (1992), measurements of water diffusion in small cylindrical pieces of apple flesh were performed {PGSE (8 samples), BGP (4 samples), and BGPR (4 samples)}. Utilizing the same pulse sequences, diffusion measurements were then performed on nerves aligned transversally or longitudinally to B_0 . Six sets of garfish nerves and ten frog sciatic nerves were used. The typical linewidths at half-height for water protons in apple and in nerve were $\sim 150 \text{ Hz}$ and 10 Hz , respectively.

Finally, in the selected cases of distilled water, apple, and the excised sciatic nerve of the frog, diffusion measurements were carried out at a much shorter diffusion time ($\delta = 3 \text{ ms}$, $\Delta = 3 \text{ ms}$ giving $t_d = 2 \text{ ms}$) in order to verify the changes in diffusion coefficient brought about by the membrane restricted diffusion orthogonal to the nerve axis. The BGP pulse sequence was used in this case and TE remained at 60 ms. To

maintain an equivalent phase wrap at these shorter timings, the transverse and longitudinal diffusion-sensitizing gradient strengths were increased to 64 G cm^{-1} and 72 G cm^{-1} , respectively.

3.2.2.3 CPMG-PGSE Pulse Sequence

The methodology presented in this section is relevant to the discussion in Chapter 5. A Stejskal-Tanner spin-echo diffusion sequence modified with a CPMG sub-sequence (Carr 1954, Meiboom 1958) (Fig. 3.14), in order to allow incrementation of the spin-echo time, was used to measure the diffusion coefficient (D_{NMR}). The $1331 \pi/2$ and 2662π binomial rf pulses were used as previously described. The gradient pulse timings ($\delta=6 \text{ ms}$, $\Delta=30.128 \text{ ms}$) were kept constant while the gradient strength, G_i , was incremented to a maximum of $\sim 12 \text{ G cm}^{-1}$ to yield a maximum gradient factor, b_{max} , of $\sim 1 \times 10^5 \text{ s cm}^{-2}$. The spacing between successive hard π rf pulses was 5 ms . The number of hard π rf pulses (n) used was 2, 18, 38, 58, and 78 to yield spin-echo times (TE) of 70, 150, 250, 350, and 450 ms, respectively. The minimum (70 ms) and maximum (450 ms) TE times were restricted by the limitations of the gradient hardware and the available signal-to-noise ratio, respectively. Echoes were averaged over 4, 8, or 16 scans, depending on the available signal to noise, with a repetition interval of 5 s (20 s for the distilled water sample). At each spin-echo time, D_{NMR} values were obtained from one component fits (5 data points per D_{NMR}) of the diffusion curves $\{\ln[(\text{echo amplitude}, G_i \neq 0) / (\text{echo amplitude}, G_i = 0)] \text{ versus } b\}$ measured both parallel and perpendicular to the long axis of the "cylindrical" sample.

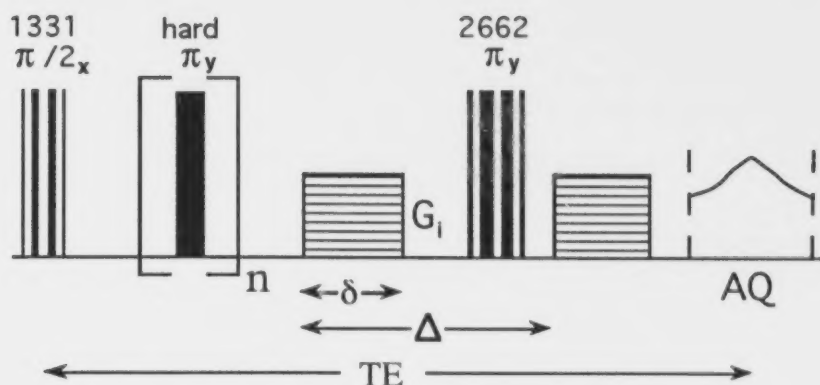


Figure 3.14 CPMG-PGSE diffusion pulse sequence in which the gradient strength (G_i) is incremented, the timings δ and Δ represent constant time intervals. TE is increased by incrementing the number of intervening hard π pulses (n). The acquisition time (AQ) for the echo peak is 10 ms.

To verify the accuracy and isotropy of D_{NMR} over a range of spin-echo times, NMR self-diffusion measurements of distilled water were performed (see Fig. 5.1 in Chapter 5). As expected, diffusion was isotropic (i.e. $D_{\text{NMR}}(\parallel) = D_{\text{NMR}}(\perp)$) and the mean D_{NMR} value was within 5% of the expected self-diffusion coefficient of pure water at 20 °C (Mills 1973). For the CPMG-diffusion pulse sequence, we also verified that the spin-echo amplitudes diminished at various TE in accordance with T_2 . For example, a routine CPMG measurement yielded a T_2 (tap water) of 2150 ms whereas CPMG-diffusion pulse sequence measurements at various TE (with $G_i = 0$) yielded a T_2 (tap water) of 2140 ms.

3.3 Methodology for Measurements of Relaxation and Magnetization Transfer

This section deals mainly with the experimental methods that are pertinent to Chapter 7. The samples were usually placed at the bottom of a 5 mm outer diameter NMR tube and the height of the sample was kept short (< 0.6 cm) so that the sample remained within the homogeneous region of the solenoidal rf coil (1 cm tall, 0.7 cm diameter). The samples of nerve were immersed in paraffin oil, as previously described in Section 3.1.1.3. The experiments were performed on a 2.35 T Bruker

CXP spectrometer. The AMT rf amplifier was used in continuous wave mode. All measurements were made at room temperature ($\sim 20^\circ\text{C}$).

3.3.1 Measurements of Transverse Relaxation Times

For samples that were immersed in paraffin oil, the transverse relaxation decay curves were measured using a phase cycled CPMG sequence (Section 2.1.4) with 1024 echoes and an inter-echo spacing of 6.4 ms. Therefore, the CPMG curve spanned from 6.4 ms to 6.6 s. The phase cycling was $+x -x (\pi/2)$, $+y -y -y +y (\pi)$, and $+AQ -AQ$. To eliminate the paraffin oil signal, the $\pi/2$ rf pulse was a 1331 binomial rf pulse with component lengths that were multiples of $1.25\ \mu\text{s}$ with binomial component separations of 1.316 ms. The rf frequency was set at the frequency of the water proton resonance. The hard π rf pulses were $20\ \mu\text{s}$ in duration. Usually, sixteen averages were obtained with a repetition interval of 10 s. For samples that were not immersed in paraffin oil, the inter-echo spacing was 1.6 ms, 4096 echoes were acquired, the hard $\pi/2$ and π rf pulses had durations of $10\ \mu\text{s}$ and $20\ \mu\text{s}$, respectively, and the repetition time was 20 s. The data were often processed by single exponential fitting of the data to yield a single T_2 value, rather than using a nonnegative least squares technique with smoothing to yield T_2 spectra (Whittal 1989). This single component approximation was sufficient for our measurements of T_2 since only a single T_2 value was necessary to calculate the effects of direct saturation (as opposed to MT) upon the observed magnetization.

3.3.2 Measurements of Longitudinal Relaxation Times

T_1 was measured using an inversion recovery pulse sequence as follows (Menon 1990),

$$0^\circ - \tau - \pi/2_x - (+AQ) - TR - \pi_{\pm y} - \tau - \pi/2_x - (-AQ) - TR \quad .$$

This technique yields a positive magnetization recovery curve starting at $2M_0$ (M_0 is the equilibrium magnetization) and dropping to zero. The inversion times (τ) were 1, 25, 100, 250, 500, 1000, and 2000 ms (i.e. 7 data points). The hard $\pi/2$ and π rf pulses had durations of $10\ \mu\text{s}$ and $20\ \mu\text{s}$, respectively. The repetition time (TR) was

10 s for nerve (and 20 s for other samples such as the agar gels). The acquisition time of the FID was 410 ms (size = 4 K, dwell time = 100 μ s). Typically, four scans were averaged. The resultant FID was Fourier transformed (FT), magnitude corrected, and the amplitudes of the water peaks were recorded. The acquisition and FT of the FID allowed us to separate spectroscopically the paraffin oil proton signal (when present) from the water proton signal, and hence, remove any influence of paraffin oil on the T_1 values of our samples. The T_1 value was obtained from the inverse of the slope of $\ln(\text{water peak amplitude})$ versus τ . Good linear lines ($r^2 \geq 0.999$) were usually obtained from our T_1 measurements.

3.3.3 Measurements of Magnetization Transfer

Three types of continuous-wave magnetization transfer (MT) measurements, which we refer to as (A), (B), and (C), were performed on our samples of garfish nerve and agar gels.

(A) In order to test the efficiency of magnetization transfer when utilizing various pre-irradiation rf parameters, the peak amplitude of water was measured as a function of the offset frequency from the water resonance ($\Delta\omega$) of a 5 s irradiation rf pulse with rf strengths (B_2) of 4.7, 16.5, and 72.5 μ T (see Fig. 2.10b for a schematic diagram of the MT CW pulse sequence). There was a 330 μ s deadtime between the irradiation and the $\pi/2$ rf pulses. Twenty-one off-resonance frequencies between 10 Hz and 100 kHz were utilized (i.e. 0.01, 0.1, 0.25, 0.4, 0.55, 0.7, 0.85, 1, 2.5, 4, 5.5, 7, 8.5, 10, 16, 25, 40, 55, 70, 85, 100 kHz). A large offset frequency of 500 kHz was used as a measure of the signal amplitude with no direct saturation or MT. A delay time of 5 s (20 s for agar gels) was used between the acquisition interval and the next irradiation pulse. The steady-state magnetization prepared by the irradiation pulse was read by acquiring the free induction decay (size = 4 K, dwell time = 100 μ s, acquisition time = 410 ms) after a 90 degree rf pulse (10 μ s duration). Two averages were usually obtained. By monitoring the peak amplitude of water in the spectrum, we were able to avoid signal contributions from the bathing paraffin oil which resonates 3.8 ppm from the water resonance. The amplitudes were normalized with respect to 0 at $\Delta\omega = 10$ Hz and 100 at $\Delta\omega = 500$ kHz.

Given the T_1 and T_2 of the sample of interest, Eqn. 2.69 allows an estimation of the degree of observed signal loss that is due to direct saturation, as opposed to the desired MT effect. The excellent agreement between calculated and experimental saturation curves (varying $\Delta\omega$) for aqueous solutions devoid of macromolecules, such as 0.5 mM MnCl_2 (aq) ($T_1 = 242$ ms, $T_2 = 19$ ms, $T_1 / T_2 = 12.7$) and 10 mM CuSO_4 (aq) ($T_1 = 136$ ms, $T_2 = 114$ ms, $T_1 / T_2 = 1.19$), established the rf strength calibrations (Fig. 3.15).

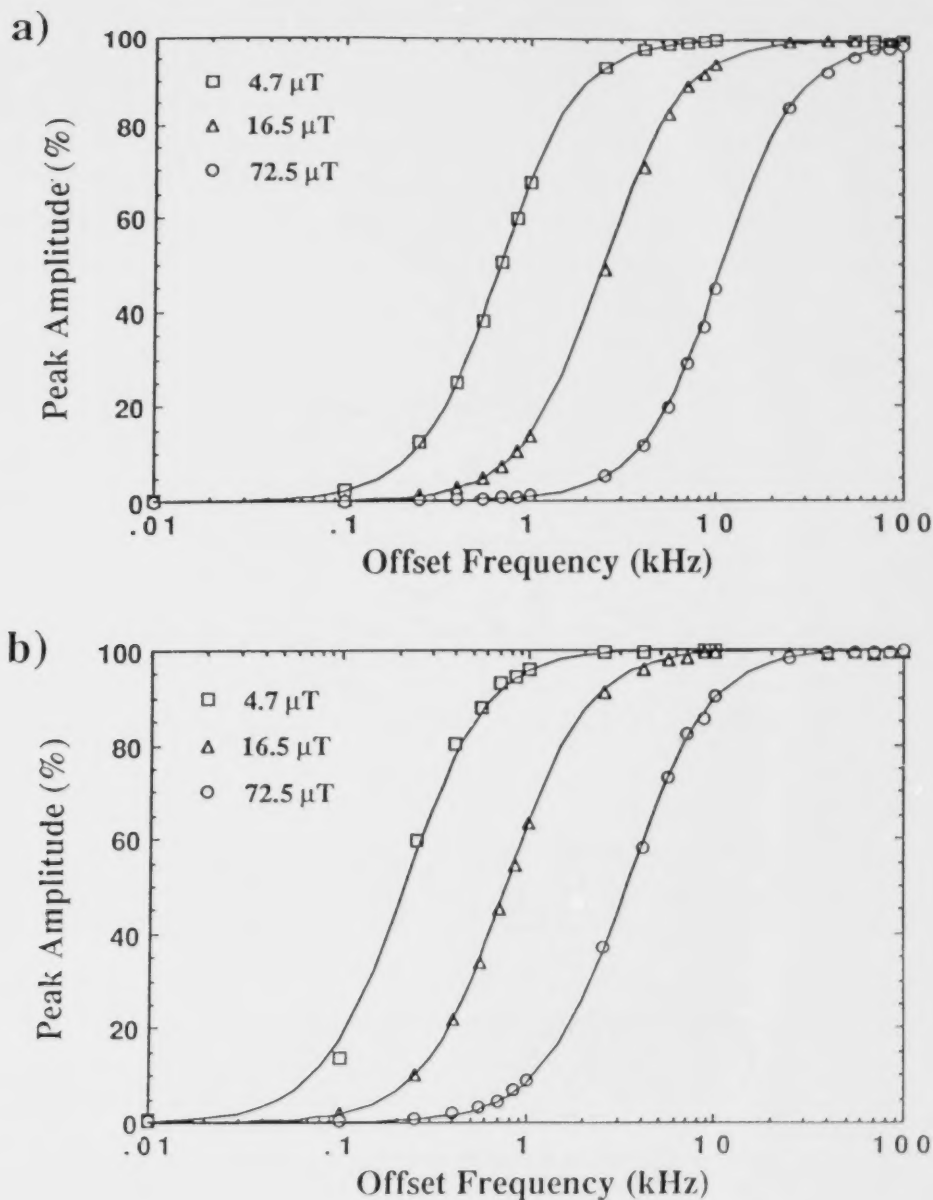


Figure 3.15 The peak amplitudes of water in 0.5 mM MnCl₂ (aq) (a) and 10 mM CuSO₄ (aq) (b) as a function of the offset frequency at irradiation rf strengths of 4.7, 16.5, and 72.5 μT are compared to direct saturation curves (solid lines) that are expected based on the T₁/T₂ of the solutions (Eqn. 2.69). The agreement between the experimental data and the calculated curves is excellent and, as expected, MT is not observed in these solutions.

(B) T_{1fsat} , the spin-lattice relaxation time of the free water protons in the presence of steady-state saturation of the macromolecular pool, was measured using a modified inversion recovery pulse sequence (Alger 1984, Eng 1991). The saturation is applied prior to the π pulse and during the inversion recovery time so that the total duration of saturation remains constant at 5 s. The off-resonance irradiation rf pulse used a $\Delta\omega$ that yielded the maximum MT effect for a particular B_2 strength. In particular, the 10 kHz, 16.5 μ T irradiation rf parameters were used since this $\Delta\omega$, B_2 combination yields the largest MT effect in our samples of gel and nerve, as will be shown later in this thesis (Chapter 7). The duration of the $\pi/2$ and π rf pulses were 10 and 20 μ s, respectively. Nine data points acquired with inversion recovery times between 2 ms and 5 s (2, 25, 100, 200, 400, 600, 1000, 2000, and 5000 ms) were used to measure T_{1fsat} . A delay time of 10 s (20 s for agar gels) between the acquisition interval and the next irradiation pulse was used for four averages. The resultant FID was Fourier transformed, phase corrected, and the amplitudes of the water peak (representing M_z) were recorded. At short inversion times, the echo peaks are negative. T_{1fsat} was obtained from the inverse of the slope of $\ln\{(M_0 - M_z)/M_0\}$ versus τ , where M_0 is the value of M_z at equilibrium ($\tau = 5$ s in our case). Assuming complete saturation of the macromolecular spins, the apparent magnetization transfer rate constant, k_{app} , of MT from the free water pool to the restricted macromolecular pool was *estimated* from the T_{1fsat} and steady-state M_{sf}/M_{of} values by Eqn. 2.66.

(C) In order to get an idea of the relative rapidity of the approach to steady-state, the peak amplitude of water as a function of the duration of the off-resonance irradiation pulse was measured using the $\Delta\omega = 10$ kHz, $B_2 = 16.5$ μ T combination of pulse parameters that yield the maximum MT for the gel and nerve samples. Fifteen different times between 0 and 7 s (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7 s) were used for the duration of the irradiation pulse. A repetition time of 5 s (20 s for gels) was used for 2 averages. Assuming complete saturation of the macromolecular spins, the dependence of the steady-state magnetization (M_{sf}), with respect to its original equilibrium magnetization (M_{of}), on the irradiation duration (t_{irr}) is given by Eqn. 2.67. Given M_{sf}/M_{of} as a function of t_{irr} , the values of T_{1fsat} , k_{app} , and T_{1f} can be calculated from Eqn. 2.67 (Forsén 1963). Alternatively, the parameters

which are independently derived from the MT measurements described in sections (A) and (B) can be verified using Eqn. 2.67.

For the aforementioned measurements (A-C), the water proton linewidths at half-maximum were $\sim 10 - 30$ Hz in the nerves and gels. The 2% gel was made by adding 0.1 g of agarose (Type 1A, Sigma) to 5 g of distilled water in a small vial. The 4% gel required the doubling of the amount of agarose. The sealed vials were heated for approximately 1/2 hour in a beaker of shallow boiling water. The vials containing the agarose were removed from the boiling water and allowed to cool (usually left overnight). The gelation is a result of the 3-dimensional network formed by the cross-linking of the polysaccharide chains of the agarose. The agar samples were placed in double-open ended NMR tubes prior to the NMR measurements. Any excess fluid adhering to the gels was removed with filter paper. Agar gels provide good samples for testing our magnetization transfer techniques and these results are provided in Chapter 7.

CHAPTER 4

Determinants of Anisotropic Water Diffusion in Nerve

Myelin was proposed as the source of anisotropic water diffusion in nerve and white matter, yet there was no conclusive experimental proof. Further, other possible sources of the anisotropy had been postulated, yet never tested. This chapter, which deals with a systematic evaluation of the potential sources of anisotropic water diffusion in nerve, is subdivided into three sections which each represent a separate published or submitted manuscript. Section 4.1 deals with the contributions of myelin, microtubules, and fast axonal transport to the observed anisotropic diffusion of water in nerve. Sections 4.2 and 4.3 deal respectively with the possible contributions of neurofilaments and background gradients to the degree of anisotropy. A description of the measurement methods and materials has been presented in Chapter 3.

4.1 The Contribution of Myelin, Microtubules, and Fast Axonal Transport to Anisotropic Diffusion¹

4.1.1 Introduction

In-vivo measurements of the voxel-average water diffusion in cat (Moseley 1990a) and human (Chenevert 1990) brain have both demonstrated marked differences between gray and white matter. Water diffusion is isotropic in gray matter whereas the average diffusion coefficients of water in white matter exhibit anisotropy. In white matter the water diffusion parallel (//) to the length of the axons was found by NMR to

¹ A version of this section has been published. C. Beaulieu, P.S. Allen, Determinants of anisotropic water diffusion in nerves. *Magn Reson Med* 31, 394-400 (1994).

C. Beaulieu, P.S. Allen, Diffusional anisotropy of water in nerve cords without myelination. *In* Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1728 (1992). Presented as a poster.

C. Beaulieu, P.S. Allen, Diffusional anisotropy of water in nerves which lack both microtubules and fast axonal transport. *In* Book of Abstracts, 12th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 605 (1993). Presented as a poster.

be more efficient than that perpendicular ($\perp$) to it (i.e. $D_{\text{NMR}}(\parallel) > D_{\text{NMR}}(\perp)$). This property has been exploited to map the myelin fiber orientations in brain non-invasively (Douek 1991).

Despite the interest in applying diffusion-weighting to MRI studies of demyelinating diseases such as multiple sclerosis (MS), little has been done to determine the structural components of white matter which impede water mobility and give rise to the anisotropy in water diffusion coefficients. Nonetheless, several possible origins have been postulated. For example, the myelin sheath enveloping the axons, the axonal membrane, and the neurofibrils (neurofilaments, microtubules) within each axon, are three longitudinally-oriented structures which could impart non-random barriers to diffusion (Le Bihan 1992), and hence decrease $D_{\text{NMR}}(\perp)$ relative to $D_{\text{NMR}}(\parallel)$. Alternatively, the water could possibly exist in an anisotropic, free liquid crystalline environment, or the measured diffusion parallel to the axons could be accentuated by axonal transport (Le Bihan 1992). Fast axonal transport is intimately linked to the presence of microtubules since cellular organelles (e.g. mitochondria, vesicles) are transported by their attachment to mechanochemical enzymes which pull the organelles along the microtubule tracks (Nicholls 1992b). However, many groups prefer the hypothesis of the myelin sheath as the primary barrier for water diffusion perpendicular to the length of the axons.

The purpose of this section is to report an investigation of the respective roles of myelin, microtubules, and fast axonal transport in anisotropic diffusion by making diffusion measurements on excised myelinated (trigeminal, optic) and nonmyelinated (olfactory) nerves from the long-nosed and spotted garfish in-vitro and by depolymerizing the microtubules required for fast axonal transport. In addition, the importance of structural integrity to anisotropic diffusion is tested by measuring diffusion in nerves that had been allowed to degenerate structurally by being maintained in refrigerated buffer for 2 months post-excision. Some of the typical structural characteristics of normal garfish nerves are given in Table 4.1.

Table 4.1 Some physical characteristics of the garfish nerves ^a.

Nerve type	Mean axonal diameter (μm)	No. of axons/nerve ^b	Space between adjacent axons ^{c, d} (μm)	No. of myelin wrappings/axon ^c
Olfactory	0.2-0.3	$\sim 10^6$ - 10^7	~ 0.03	None
Trigeminal	3-6 ^e	$\sim 10^3$ - 10^4	~ 1	~ 35 -40
Optic	1-3	$\sim 10^5$ - 10^6	~ 0.8	~ 15 -20

^a Figures 3.2, 3.3, and 3.4 contain transverse and longitudinal electron micrographs of the garfish nerves.

^b Estimated from the mean axonal and nerve fiber diameters (~ 0.3 , 0.15 , and 0.8 mm for olfactory, trigeminal and olfactory nerves).

^c These are gross estimates taken from electron microscope thin sections.

^d For myelinated nerves, the space corresponds to the distance between the myelin sheaths enveloping the adjacent axons.

^e Some axons are greater than $10 \mu\text{m}$.

4.1.2 Results

Representative parallel and perpendicular diffusion decay curves for the three types of freshly excised nerves of the garfish are shown in Fig. 4.1, clearly demonstrating anisotropy in the diffusion properties of nerve water irrespective of the presence or absence of myelin. The limiting D_{NMR} values quoted in Table 4.2 correspond to a range of b (the gradient factor is defined in Eqn. 2.46), namely $0 \leq b \leq 1 \times 10^5 \text{ s cm}^{-2}$, that is typical for clinical diffusion measurements. They were derived from a single component fitting approximation over this range. The degree of anisotropy (for the same range of b) given by the diffusion coefficient ratio $D_{\text{NMR}}(\parallel)/D_{\text{NMR}}(\perp)$ is quite similar in all three nerves. The diffusion decay curves over the range of b extending from $1 \times 10^5 \text{ s cm}^{-2}$ to $8 \times 10^6 \text{ s cm}^{-2}$ demonstrate a marked departure from a single exponential dependence of the echo amplitude on b , while at the same time retaining an anisotropic character.

In the vinblastine treated nerves the D_{NMR} values are consistently less than in their untreated counterparts (Table 4.2). Nonetheless, anisotropic diffusion is still observed and the degree of anisotropy is quite similar to the untreated case. Isotropy

(or markedly reduced anisotropy) is demonstrated only in the in-vitro degenerated nerves (Table 4.2).

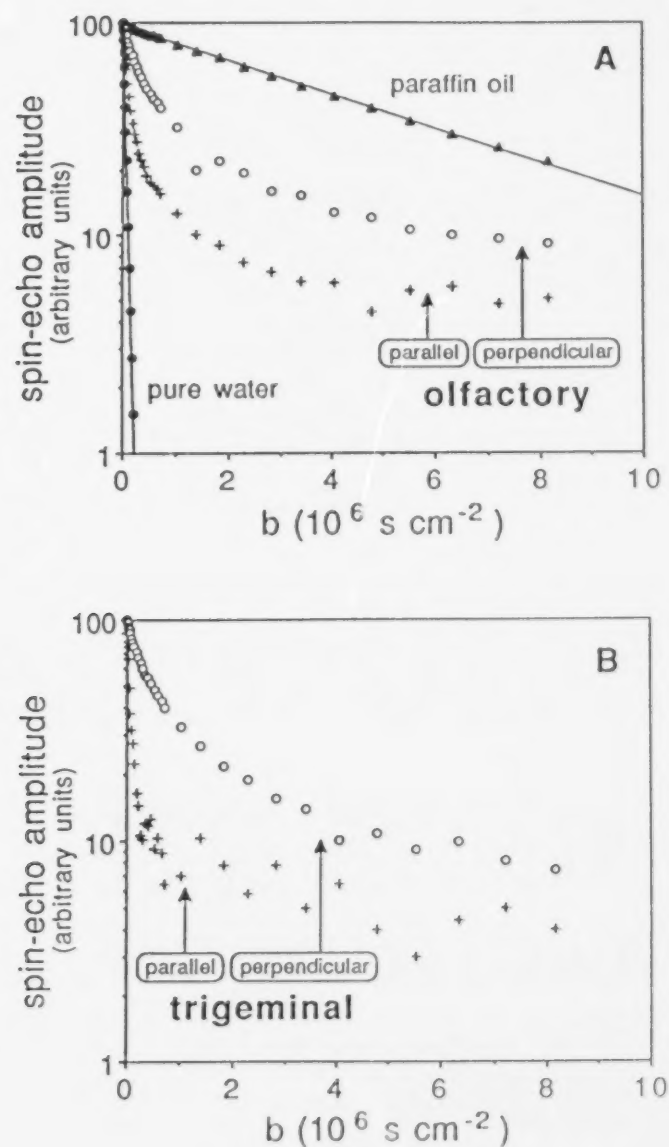


Figure 4.1 Figure caption on next page.

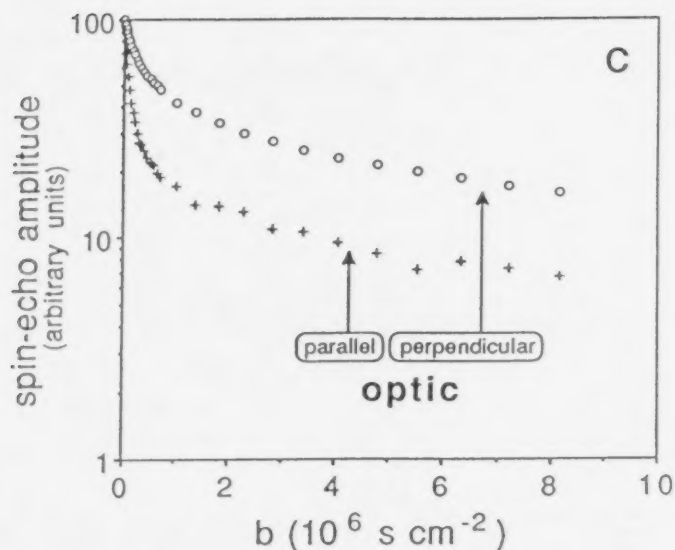


Figure 4.1 Representative diffusion decay curves for water in the freshly excised nonmyelinated olfactory nerve (A), the myelinated trigeminal nerve (B), and the myelinated optic nerve (C) of the garfish. The diffusion curves of pure water and paraffin oil are provided for comparison in (A). Parallel and perpendicular diffusion measurements refer to the long axis of the nerve fibers, and demonstrate the anisotropy of water diffusion in all three nerve types. The gradient factor, b , is defined in Eqn. 2.46. The diffusion time is 28 ms.

Table 4.2 NMR measurements of diffusion coefficients of pure water, paraffin oil, and water in freshly excised, vinblastine treated, and in vitro degenerated garfish nerves.

Samples	$D_{\text{NMR}}(\parallel)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$
Pure water at 22 °C ^a	2.11 ± 0.06	2.09 ± 0.06	1.0 ± 0.1
Paraffin oil ^{b, c}	0.021 ± 0.002	0.020 ± 0.002	1.0 ± 0.1
Garfish nerve water ^{c, d, e}			
Freshly excised (N=11)			
Olfactory	0.96 ± 0.20	0.33 ± 0.09	3.6 ± 1.2 ^f
Trigeminal	1.27 ± 0.16	0.44 ± 0.07	3.2 ± 0.9
Optic	0.65 ± 0.07	0.26 ± 0.03	2.6 ± 0.4
Vinblastine treated (N=5)			
Olfactory	0.45 ± 0.08	0.18 ± 0.03	2.6 ± 0.4
Trigeminal	0.90 ± 0.22	0.31 ± 0.08	2.8 ± 0.8
Optic	0.42 ± 0.22	0.20 ± 0.06	2.2 ± 0.7
In vitro degenerated (N=4)			
Olfactory	0.39 ± 0.16	0.36 ± 0.19	1.1 ± 0.2
Trigeminal	0.49 ± 0.32	0.37 ± 0.29	1.4 ± 0.5
Optic	0.34 ± 0.13	0.29 ± 0.19	1.3 ± 0.6

^a For a single sample measurement the error in the best fit was typically $< \pm 0.01 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. For repeated measurements the spread in D_{NMR} values was typically within 3% of the expected literature values (Mills 1973).

^b Averaged over five measurements, the errors reflecting the 95% confidence interval.

^c Measurements made at room temperature which varied slightly (20-25 °C) between each experiment.

^d The mean $\pm$ 95% confidence interval for measurements on N samples.

^e Diffusion coefficients obtained from echoes with $0 \leq b \leq 1 \times 10^5 \text{ s cm}^{-2}$.

^f The mean $\pm$ 95% confidence interval of the diffusion coefficient ratios were obtained from the ratios for the individual nerves.

4.1.3 Discussion

The observation of anisotropic water diffusion in the nonmyelinated olfactory nerve of the garfish provides, to our knowledge, the first unequivocal evidence that myelin is not an essential determinant for anisotropic diffusion in axonal systems. In other words, structural features of the axons other than myelin, are sufficient to give

rise to anisotropy, even though myelin may still contribute. The degree of anisotropy for the three nerve types studied is similar irrespective of the presence or absence of myelin (Table 4.2). Moreover, both the observed diffusion coefficients and their anisotropy ratios are similar to those obtained in-vivo for white matter (Table 2.2), thus lending some credibility to extending the conclusions gained from garfish nerve data to the human central nervous system (CNS).

The assumption that myelin is an essential determinant of anisotropic diffusion in white matter has led to a number of suggestions in the literature, which, in light of our data, may no longer be tenable. For example, the apparently conflicting findings (Rutherford 1991) of (i) diffusional anisotropy, which purportedly indicated myelin, and (ii) T_1 weighted images which indicated no myelination, can now be reconciled without postulating a greater sensitivity of diffusion weighting than of T_1 weighting to myelination in the developing brain. Our observations also indicate that for an explanation of the observed changes in diffusion coefficient components as the brain matures (Sakuma 1991), one should be prepared to look beyond the changes in the myelin and possibly to the developing cytoskeletal organization within the axons (Cancalon 1984). That sources of diffusional anisotropy exist, alternative to myelination, is also consistent with the range of findings in MS lesions (Mikulis 1988, Larsson 1990, Forti 1991, Segawa 1992b, Larsson 1992), where the appearance of diffusion-weighted MR images does not seem to have been conclusive in diagnosing demyelination.

Another postulated source of the anisotropy (Le Bihan 1992) and one which can be tested experimentally, is that of fast axonal transport, although previous doubts (Doran 1990) had been cast on the significance of this mechanism, based on typical axonal transport velocities. Microtubules and microtubule-associated proteins are required for fast axonal transport (Nicholls 1992b), and by depolymerizing the microtubules with vinblastine, fast axonal transport can be inhibited. The data presented in Table 4.2 are, to our knowledge, the only demonstration of anisotropic water diffusion in nerves whose microtubules have been depolymerized (Figs. 4.2 and 4.3)². This demonstration of continued anisotropy shows that microtubules, of themselves, and the fast axonal transport they facilitate are not the dominant

² The reader is referred to Figs. 1 and 26 in Smith 1975a, which show electron microscopy sections of axons before and after vinblastine treatment and demonstrate the elimination of microtubules.

determinants of anisotropy. Both parallel and perpendicular components of the diffusion coefficient are reduced in vinblastine treated nerves, relative to their freshly excised values. These reductions in D_{NMR} may be due to the presence of vinblastine paracrystals (also called microtubule crystals), to a cytoplasmic increase in free tubulin (the monomeric unit of microtubules), and/or to some axonal degeneration (e.g. axonal shrinkage). Although the anisotropy ratios appear to be reduced by vinblastine treatment, they are still significantly different from unity and moreover, the freshly excised and vinblastine treated anisotropy ratios fall within the limits of their respective errors for all three garfish nerves.

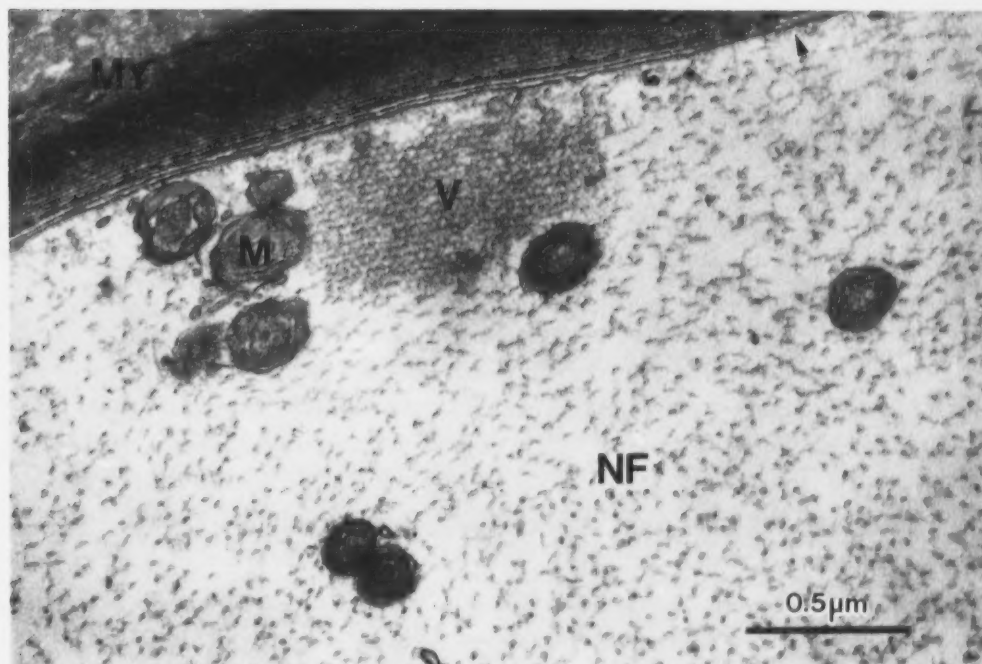


Figure 4.2 Transverse electron microscopy section of part of an axon in a garfish trigeminal nerve that has been immersed for 48 h in refrigerated buffer containing 0.5 mM vinblastine sulfate. No intact microtubules are evident whereas a large number of neurofilaments (NF) seem unaffected by the treatment. The regular, hexagonal, honeycomb patterns (V) observed in the axoplasm are the result of the vinblastine treatment and are referred to as "vinblastine paracrystals" (Smith 1975) or "microtubule crystals" (Bensch 1969). A portion of the myelin sheath (MY), the axonal membrane (arrowhead), and the mitochondria (M) are visible.

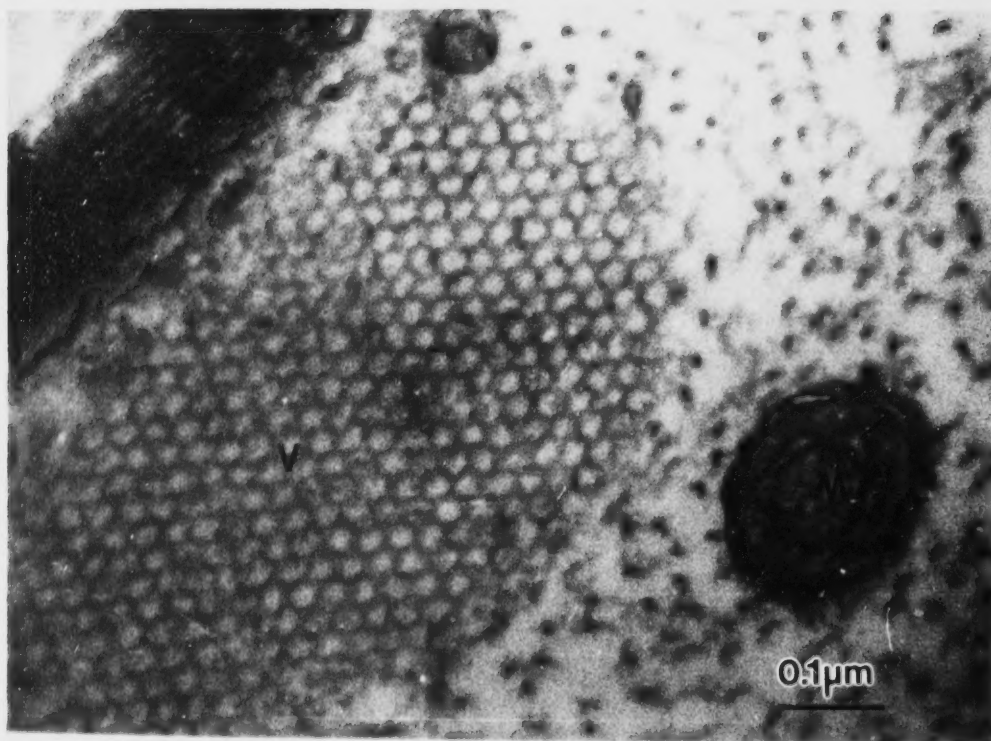


Figure 4.3 Electron microscopy closeup of "vinblastine paracrystals"/"microtubule crystals" (V) in an axon. A mitochondrion is visible (M).

With regard to the remaining structures, one might first obtain a rough estimate of the significance of axonal membranes by considering the anisotropy values of the three nerves in relation to their respective axonal diameters. Assuming similar permeabilities for their axonal membranes, one might anticipate that if this membrane were a significant hindrance to water diffusion, then water molecules traversing the greatest number of membranes would show the greatest anisotropy. Typical axonal diameters vary by at least a factor of ten between the nonmyelinated olfactory nerve on the one hand and the larger axons of the myelinated trigeminal and optic nerves on the other hand (Table 4.1). However, the observed anisotropies vary little between nerve types and this might suggest that the axonal membrane is but a minor player in the origin of anisotropy. However, one must not lose sight of a possible explanation for

the apparent lack of an axonal size effect and one which supports a significant role for membranes. For example, one could postulate that in a given diffusion time, a water molecule would encounter a similar number of lipid bilayers in the nonmyelinated nerve with smaller axons (i.e. as axonal membranes) as it would in the myelinated nerves with larger axons (i.e. as myelin wrappings). To obtain a crude *relative* estimate of the number of membranes that are traversed during a diffusion time, t_d , we can determine how many axons fall along a line equal in length to the root-mean-square diffusional displacement, λ_1 , in the garfish nerves, where

$$\lambda_1 = (2 D_{\text{NMR}} t_d)^{1/2}$$

for diffusion in one dimension. For water in the freshly excised garfish nerves, parallel and perpendicular estimates for λ_1 are listed in Table 4.3. These values were estimated using $t_d = 28$ ms and the measured one-component diffusion coefficients quoted in Table 4.2. By comparison, a t_d of 28 ms would yield an isotropic λ_1 of ~ 11 μm for pure water. A water molecule traveling a distance λ_1 directly from its origin in a transverse direction would traverse between 30 and 40 membranes in the olfactory nerve, between 30 and 70 membranes in the trigeminal nerve, and between 20 and 70 membranes in the optic nerve, based on the range of axonal diameters and number of myelin wrappings listed in Table 4.1. These rudimentary estimates would suggest that a significant role for membranes, either in the form of myelin or the axonal membrane, is not inconsistent with the diffusional anisotropy data presented in Table 4.2.

Table 4.3 One-dimensional root-mean-square displacements, λ_1 , for the diffusion of water parallel (//) and perpendicular ($\perp$) to the axonal length in freshly excised garfish nerves over a diffusion time of 28 ms.

Nerve	$\lambda_1(\text{//})$ (μm)	$\lambda_1(\perp)$ (μm)	Estimated No. of membranes in a path length $\lambda_1(\perp)$ ^a
Olfactory	7.3 ± 0.8	4.3 ± 0.6	28 - 44
Trigeminal	8.5 ± 0.6	5.0 ± 0.4	28 - 68
Optic	6.0 ± 0.4	3.8 ± 0.2	20 - 76

^a Determined in conjunction with data from Table 4.1.

By eliminating a dominating role for fast axonal transport and microtubules, and by acknowledging the uncertain role of axonal membranes and myelination as the sole determinants of anisotropic diffusion, one is led to consider the significance of the role of the neurofilamentary cytoskeleton. For example, in the dense axonal cytoskeleton a uniform neurofilamentary density of typically 400 per μm^2 is maintained, with neighbouring neurofilaments (diameter ~ 10 nm) being separated by 20-50 nm (as seen in Fig. 4.2). In the larger axons neurofilaments greatly outnumber the microtubules (diameter ~ 25 nm) which typically congregate near mitochondria, whereas in smaller axons neurofilaments and microtubules generally have comparable populations. The numerous longitudinal channels formed by the neurofilaments are potentially favourable to translational displacement parallel to the channels, since water molecules will encounter a very large number of extended neurofilaments when moving perpendicular to the nerve axis during the diffusion time.

The importance of intact longitudinal structures in generating anisotropy is consistent with the markedly reduced anisotropy (often isotropy) observed in garfish nerves which have been allowed to degenerate at a low temperature post-excision (Table 4.2). Interestingly, $D_{\text{NMR}}(\perp)$ in the degenerated nerves is found to be similar to that measured in the freshly excised nerves. The reduction of $D_{\text{NMR}}(\parallel)$ from its value in the freshly excised nerves to a value similar to $D_{\text{NMR}}(\perp)$ indicates an isotropic, spatial redistribution of cellular debris due to the breakdown of the cytoskeleton and various membranes in the degenerated nerves. However, one must realize that the opposite pattern of changing D_{NMR} values, namely, $D_{\text{NMR}}(\perp)$ increasing to $D_{\text{NMR}}(\parallel)$ which, in turn, remains relatively constant could occur *in-vivo* (Segawa 1993) because of macrophage removal of the broken down cellular debris in the *in-vivo* degenerating nerve. Our observation that a slight anisotropy is still present in some of the *in-vitro* degenerated nerves ($D_{\text{NMR}}(\parallel)/D_{\text{NMR}}(\perp)$ ranged from 1.0-1.3 for the olfactory nerve, 0.9-1.7 for the trigeminal nerve, and 1.0-1.7 for the optic nerve) may reflect incomplete degeneration of some of the longitudinal structures.

Although axonal structures have been involved in the foregoing discussion to explain diffusional anisotropy, the water in these structures, namely the axoplasmic water and the myelin water, does not usually exceed $\sim 70\%$ of the total water in the garfish nerves studied (Fenrich 1991, Fenrich 1992). The remaining fraction (which

varies between nerve types) resides in the glia and in the extracellular spaces. The effect which this potential compartmentalization of water might have on the diffusion decay curves is not easy to determine from our limited data. Other groups (van Zijl 1991, Laicher 1992, Kwong 1994) have postulated water compartmentalization as the source of the curvature in their semi-logarithmic diffusion decay curves. We also observed a marked curvature, as well as an anisotropy, in the diffusion decay curves shown in Fig. 4.1. Nonetheless, there are other possible sources of this curvature. Restricted diffusion (Karger 1988b, Zhong 1991a) is undoubtedly one and some of the axonal structures described previously could potentially provide such a restrictive influence. The dimensions are appropriate. A good example of the multiexponential nature of the diffusion curve as a result of restricted diffusion perpendicular to a series of parallel plates is reported in Fig. 1 of Hürlimann et al. (1995).

The diffusion decay curves (parallel and perpendicular diffusion) (Fig. 4.1) could often be fitted to three exponentials with "D" values $\sim 1 \times 10^{-5}$, 0.15×10^{-5} , and $0.010 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ with similar contributions for all three nerve types. This similarity between all three nerves precluded a correlation between the number of diffusion components and the individual water compartments, as is often performed with T_2 analyses of nerve (Fenrich 1991, 1992, Menon 1991, 1992). To evaluate whether or not there was additional information (i.e. about water compartmentation) in the diffusion curves of the garfish nerves (Fig. 4.1), the diffusion data was processed to yield $\mathbf{q}$ -space distributions of the particle displacements, as described in Section 2.1.5.4. The original diffusion decay curves (32 data points) were interpolated to yield equally spaced data points with respect to $\mathbf{q}$, zero-padded to 256 data points, and then Fourier transformed to yield the displacement probabilities (Fig. 4.4). The displacement curves in $\mathbf{q}$ -space are very similar for all three nerves, and therefore, $\mathbf{q}$ -space does not aid in discriminating between the nerves. Others have shown differences in the $\mathbf{q}$ -space displacement profiles between normal and ischemic rat brain, and suggest that this analysis method will be useful in diffusion-weighted imaging of brain tissue (King 1994). However, in my opinion, the $\mathbf{q}$ -space analysis (with respect to diffusion) of these biological tissues, including our garfish nerves, does not provide any beneficial information that is not already obtained from the simpler measurement of single-component diffusion coefficients from $\ln(\text{echo amplitude})$ versus b .

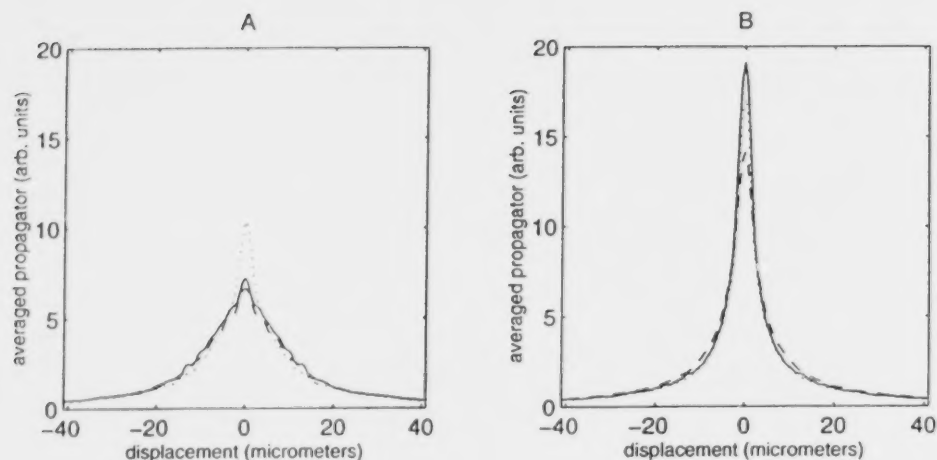


Figure 4.4 q-space representations of probabilities of water displacements parallel (A) and perpendicular (B) to the long axis in the olfactory (solid), trigeminal (dashed), and optic (dotted) nerves of the garfish. As expected for anisotropic diffusion, greater displacements are observed in the parallel direction than in the perpendicular direction.

4.1.4 Conclusion

Using garfish nerves as a CNS model, we have established that anisotropic water diffusion is still present in nerves which lack myelin, which lack microtubules, and which lack fast axonal transport and moreover, to a degree which is similar to that in nerves which contain them. This is not to say, however, that myelin does not contribute to any observed anisotropy, only that it is not a prerequisite in some axonal systems. Other longitudinally-oriented barriers to diffusion such as the axonal membrane or the extensive neurofilamentary cytoskeleton are shown to be sufficient to generate diffusional anisotropy. The relative contributions of these other longitudinal barriers are still unknown. Anisotropy of water diffusion cannot therefore be expected to be a *specific* marker of myelination in white matter.

4.2 The Contribution of Neurofilaments to Anisotropic Diffusion³

4.2.1 Introduction

The highly-oriented structure of nerve and white matter leads to diffusional characteristics of their water protons, as measured by NMR, which are different from those measured in either muscle or gray matter. Marked diffusional anisotropy is observed in white matter (Moseley 1990a, Chenevert 1990) and in excised nerve (Section 4.1). For example, the diffusion coefficient (D_{NMR}) parallel ($\parallel$) to the length of the axons is two to four times greater than that perpendicular ($\perp$) to it. In a study of the potential origins of this anisotropy, it was found for excised garfish nerve (Section 4.1) that myelin was not essential for diffusional anisotropy and that neither the presence of microtubules nor the concomitant fast axonal transport were a requirement for such anisotropy either. In this previous study, the possible translational hindrances producing anisotropy were reduced to either the membranes, in the form of myelin or multiple axonal membranes, or to the longitudinally-oriented neurofilamentary cytoskeleton within the axoplasm. The purpose of the present work is to evaluate the influence of the neurofilamentary cytoskeleton on water mobility by making measurements in axoplasm with minimal interference from membranes.

Because of their large diameter (~ 200 to $1000 \mu\text{m}$) the giant axons found in squid (Young 1936, Young 1938) (Fig. 4.5) provide an axoplasmic space whose dimensions are much greater than the one-dimensional root-mean-square displacement ($\sim 11 \mu\text{m}$) of a water molecule randomly diffusing over 30 ms, a typical diffusion time for NMR studies. Hence, very few water molecules, relatively speaking, will encounter the axonal membrane during the diffusion measurement time. Consequently, the degree of obstruction by the longitudinal neurofilaments, the primary structural component of the axoplasm, can be determined by measuring D_{NMR}

³ A version of this section has been published. C. Beaulieu, P.S. Allen, Water diffusion in the giant axon of the squid: implications for diffusion-weighted MRI of the nervous system. *Magn Reson Med* 32, 579-583 (1994).

C. Beaulieu, P.S. Allen, Water diffusion in the giant axon of the squid. In Book of Abstracts, 2nd Annual Meeting of the Society of Magnetic Resonance, San Francisco, p. 440 (1994). Presented as a talk.

of axoplasmic water, and the relative importance of the neurofilamentary cytoskeleton, on the one hand, and the membranes, on the other, as the source of anisotropic water diffusion, can be assessed. In addition, the experimental results of diffusion in the squid giant axon were also compared to "Monte Carlo" computer simulations of two-dimensional diffusion amongst a regular, hexagonal array of circular barriers which represent the neurofilaments present in the axoplasm.

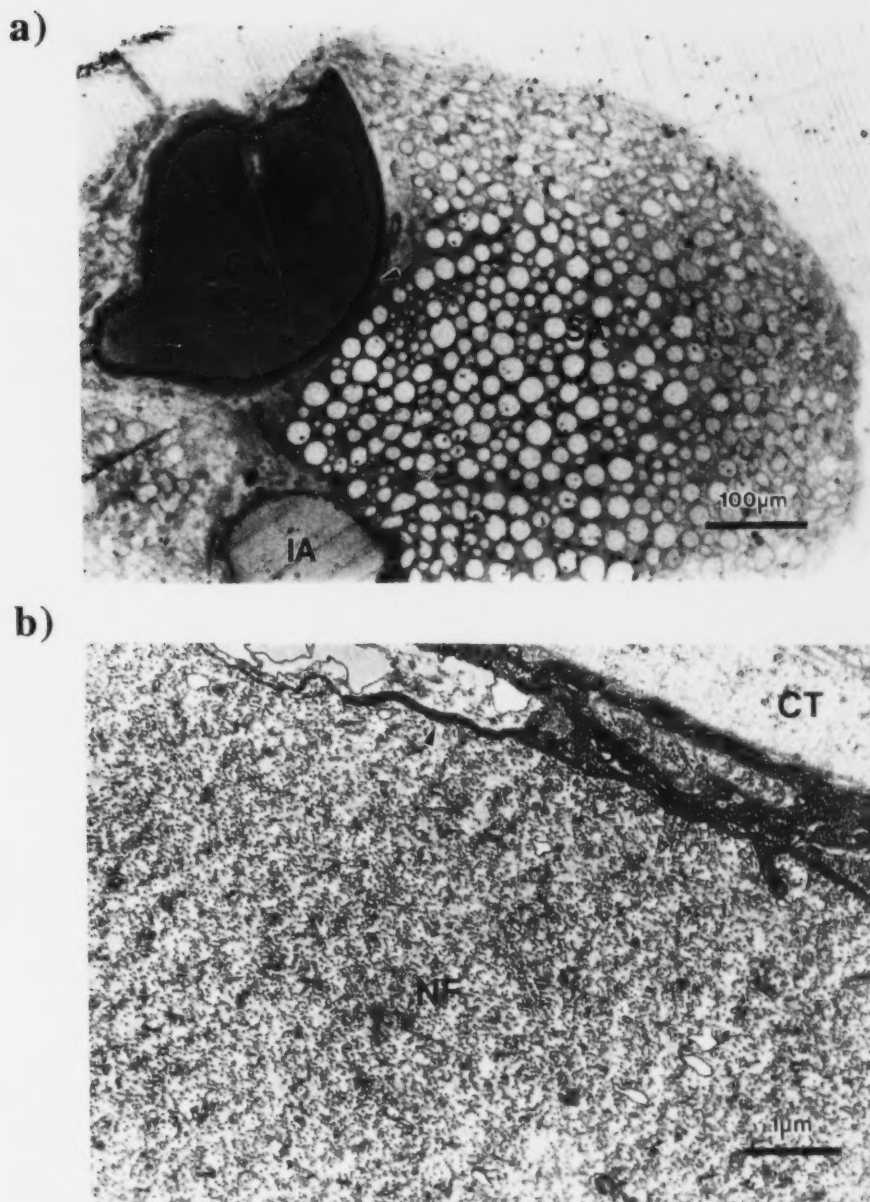


Figure 4.5 (a) Light microscopy of a transverse section of the hindmost stellar nerve from a squid. The giant axon (GA) is surrounded by Schwann cells and connective tissue (arrowhead), and smaller- (SA) and intermediate- (IA) sized axons. (b) Electron microscopy of a portion of the interior of a giant axon from a squid. Numerous neurofilaments (NF) (seen as small black dots) are visible within the axoplasm. The axonal membrane (arrowhead), Schwann cells (SC), and connective tissue (CT) are also visible.

4.2.2 Materials and Methods

Section 4.2 is comprised of results from diffusion measurements of the squid giant axon and computer simulations of diffusion in a model of neurofilaments.

The dissection procedure of the squid giant axon and details of the diffusion measurements have been presented in Section 3.1.2. A light micrograph of the stellar nerve that contains the squid giant axon and an electron micrograph of the interior of the squid giant axon are shown in Fig. 4.5. The computer simulation procedure has not been discussed previously and is therefore described in the following paragraphs.

To simulate the degree of hindrance that a longitudinally-oriented neurofilamentary lattice might have on the translational displacement of water molecules perpendicular to the long axis of the neurofilaments, "Monte Carlo" calculations of two-dimensional diffusion amongst an extensive hexagonal array of impenetrable circular barriers (Fig. 4.6) were carried out and were compared to two-dimensional diffusion in the absence of the barriers. A hexagonal array was chosen so that each barrier was equidistant from all of its nearest neighbours. The diameter (d) of the circular barriers was chosen to be 10 nm (typical neurofilament diameter), and regular, hexagonal arrays were chosen with lattice-spacings (R) of 60, 30, 25, and 20 nm (illustrated in Fig. 4.7) spanning the range found in axons (Hirokawa 1991). Computer simulations were performed on 10,000 non-interacting, diffusing particles, starting at the origin and using a step size of $1/5$ of the separation between the edges of the circular barriers (when present). The number of steps (in the given diffusion time) was adjusted to produce the analytical root-mean-square (RMS) displacement, λ , of the particles when diffusing freely without barriers. Unhindered diffusion was chosen to represent the typical $D(//)$ in white matter and in nerves, i.e. $D_{\text{no barriers}} = 1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, giving rise to λ for one-dimensional and two-dimensional unhindered diffusion of $7.8 \text{ } \mu\text{m}$ $\{\lambda_1 = (2Dt_d)^{1/2}\}$ and $11.0 \text{ } \mu\text{m}$ $\{\lambda_2 = (4Dt_d)^{1/2}\}$, respectively, for a diffusion time, t_d , of 30 ms. In order to obtain a viable computational time, a factor of 10 scaling was used in the absence of barriers. Maintaining the same parameters in the presence of barriers (except that if a particle moved within the boundary of one of the circular barriers after a step, the particle was returned to its original position prior

to that step) the final particle positions and the standard deviations of the displacements in one- and two-dimensions were determined. The quadratic relationship between diffusion coefficient and RMS displacement (Eqn. 2.30) can then be used to obtain the anisotropic diffusion ratios from the ratios of λ^2 in the absence {assumed to be proportional to $D(\parallel)$ } and in the presence {i.e. proportional to $D(\perp)$ } of barriers.

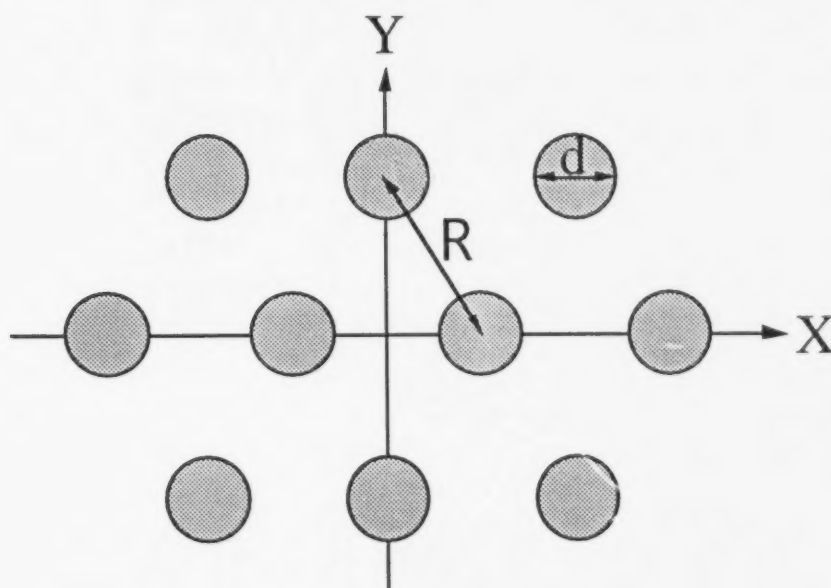


Figure 4.6 A regular, hexagonal array of circular barriers with diameter, d , and lattice spacing, R , is illustrated. In order to realistically model diffusion amongst the neurofilaments in the axoplasm, $d = 10$ nm and $R = 60, 35, 25, 20$ nm. The array of barriers is present over the entire XY space that is sampled by the particles undergoing the random walk.

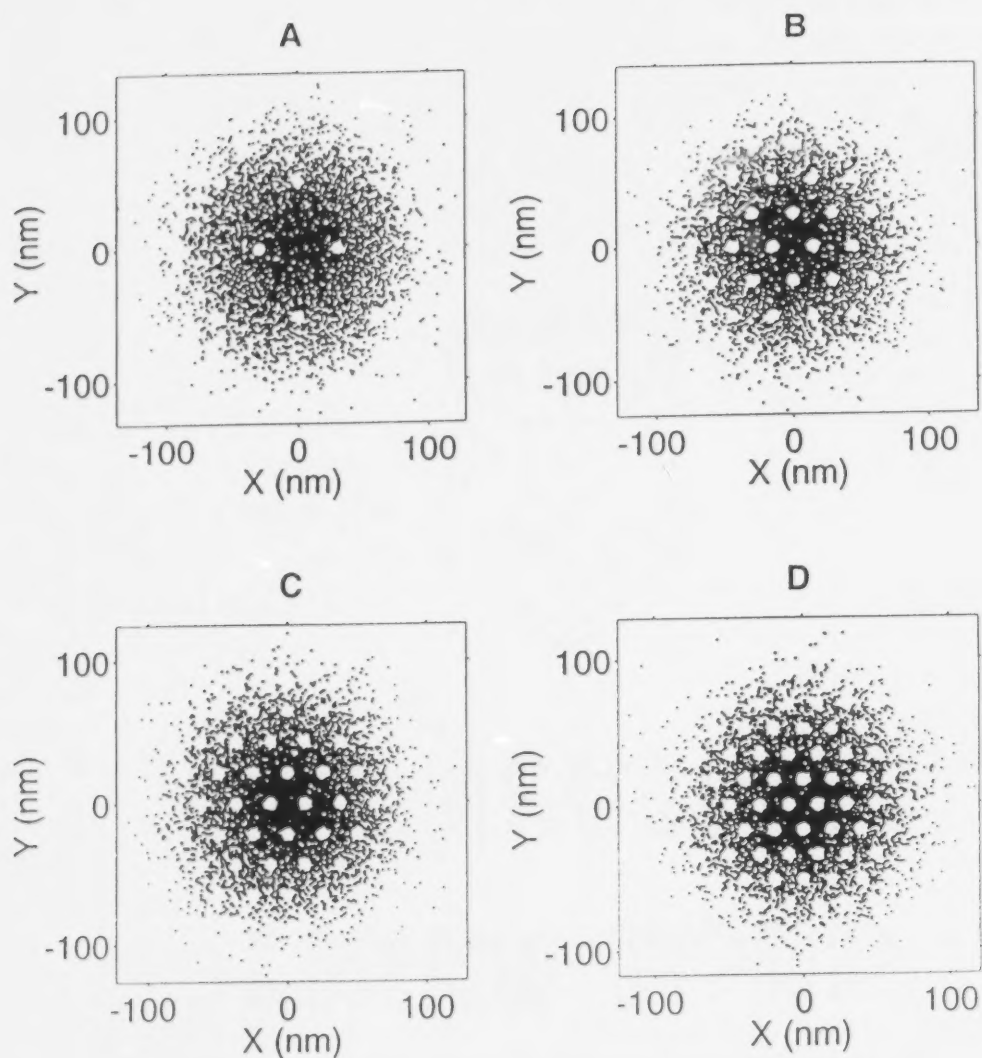


Figure 4.7 Illustration of the lattice spacings of 60 (A), 30 (B), 25 (C), and 20 nm (D) for the 10 nm diameter circular barriers which are arranged in a hexagonal array. 10,000 particles originally positioned at the origin ($X = 0$ nm, $Y = 0$ nm) were allowed to randomly diffuse for 100 steps, and their final positions are shown.

The efficacy of the simulation was indicated by the square root dependence of λ on the number of steps, the inverse square relationship between step size and the number of steps to maintain a fixed λ in the absence of barriers, and the Gaussian nature of the distributions of one-dimensional displacements along any particular axis, (Figs. 4.8B, 4.8E).

4.2.3 Results

$D_{\text{NMR}}(\parallel)$, $D_{\text{NMR}}(\perp)$, and the anisotropic diffusion ratios $\{D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)\}$ for water in fifteen squid giant axons are given in Table 4.4. A slight non-monoexponential behaviour (less than 5% amplitude for a second component) is observed if the perpendicular diffusion curve is taken to large b ($\sim 8 \times 10^6 \text{ s cm}^{-2}$). The single-component transverse relaxation time (T_2) for water in the giant axons (immersed in paraffin oil to avoid fast exchange with the buffer) is $170 \pm 30 \text{ ms}$.

The results of the computer simulations are shown in Table 4.5 where the root-mean-square displacements (λ) and the calculated anisotropic diffusion ratios are given for water particles randomly diffusing in the absence or presence of a regular, hexagonal array of circular barriers. Typical spatial distributions for the diffusion of particles in the absence (A-C) and presence (D-F) of barriers (with a lattice spacing of 20 nm, for example) are shown in Fig. 4.8.

Table 4.4 NMR measurements of water diffusion parallel (//) and perpendicular ($\perp$) to the long axis in the giant axon of the squid.

Squid Giant Axon Sample No.	$D_{\text{NMR}}(\text{//})^a$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)^a$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\text{//})}{D_{\text{NMR}}(\perp)}$
1	1.48	1.47	1.0
2	1.68	1.33	1.3
3	1.51	1.12	1.3
4	1.71	1.05	1.6
5	1.52	1.24	1.2
6	1.70	1.54	1.1
7	1.60	1.57	1.0
8	1.69	1.35	1.3
9	1.50	1.26	1.2
10	1.80	1.40	1.3
11	1.65	1.49	1.1
12	1.44	1.29	1.1
13	1.52	1.40	1.1
14	1.77	1.34	1.3
15	1.57	1.12	1.4
All samples ^b	1.61 ± 0.06	1.33 ± 0.09	1.2 ± 0.1

^a Measurements made at room temperature ($20 \pm 1^\circ\text{C}$).

^b The mean $\pm$ 95% confidence interval.

Table 4.5 The root-mean-square displacements in one- and two-dimensions (λ_1 and λ_2 , respectively), and the calculated diffusion coefficient anisotropy for the computer simulations of random diffusion in the absence and presence of a regular, hexagonal array of impenetrable circular barriers (details are given in the main body of the text) ^a.

Step size (nm)	No. of steps	Without barriers		With barriers ^b			Diffusional Anisotropy ^{c,d,e}
		λ_1 (μm)	λ_2 (μm)	Lattice spacing (nm)	λ_1 (μm)	λ_2 (μm)	
10	12,100	0.78	1.10	60	0.76	1.08	1.05
4	75,625	0.78	1.10	30	0.73	1.03	1.14
3	134,443	0.78	1.11	25	0.70	0.99	1.24
2	302,500	0.78	1.10	20	0.68	0.96	1.32

^a Each simulation used 10,000 randomly diffusing particles.

^b A diameter of 10 nm for the circular barriers corresponds to the diameter of the cross-section of a neurofilament.

^c We assume that the presence of the longitudinally oriented barriers does not impede diffusion along the axis of the barriers, and hence $D(\parallel) = D_{\text{no barriers}}$.

^d $[\lambda_1(\text{no barriers})]^2 / [\lambda_1(\text{barriers})]^2$ is proportional to $D(\parallel) / D(\perp)$.

^e One can use either the one- or two-dimensional RMS values.

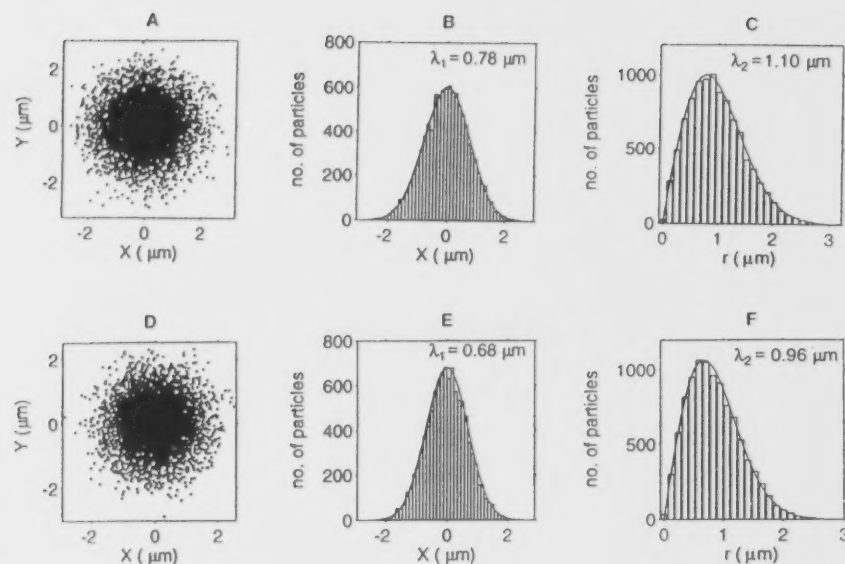


Figure 4.8 Typical results for random-walk simulations of two-dimensional diffusion in the absence {(A) - (C)} and presence {(D) - (F)} of a regular, hexagonal array of circular barriers ($d = 10$ nm, $R = 20$ nm). The final particle positions after two-dimensional diffusion (step size = 2 nm, number of steps = 302,500) are shown for 10,000 particles that originated at $X = 0$ μm , $Y = 0$ μm (A, D). As expected, the histogram for the number of particles at various X locations along $Y = 0$ μm , for example, is in good agreement with a simulated Gaussian curve (B, E). The histogram for the number of particles at various distances, r , $\{r_i = (X_i^2 + Y_i^2)^{1/2}$ for particle i from the origin is shown (C, F). For this lattice spacing ($R = 20$ nm), λ_1 and λ_2 have decreased by $\sim 13\%$ in the presence of barriers (E, F) with respect to diffusion without barriers (B, C).

4.2.4 Discussion

It is clear from Table 4.4 that water diffusion in the axoplasm of the giant axon of the squid is rapid and only weakly anisotropic. $D_{\text{NMR}}(\parallel)$ and $D_{\text{NMR}}(\perp)$ have decreased by $\sim 20\%$ and $\sim 34\%$, respectively, from the D value of pure water. The

small magnitude (14%) of the reduction of $D_{\text{NMR}}(\perp)$ with respect to $D_{\text{NMR}}(\parallel)$ shows that the longitudinally-oriented neurofilaments are unable to provide sufficient hindrance to water diffusion to account for the marked anisotropy of water diffusion observed in excised nerves and in white matter. They are not therefore, thought to be a primary determinant for that marked anisotropy.

The residual anisotropy which is observed in the axoplasm of the squid giant axon can be explained by simulating the diffusion of water molecules amongst an hexagonal array of circular barriers which represent the neurofilaments in the axoplasmic space. However, one must bear in mind that local gradients in the transverse z direction due to the susceptibility differences in the inhomogeneous system may well have enhanced the observed anisotropy (Hong 1992). If this is the case, then the role of neurofilaments in producing diffusional anisotropy is even less than can be readily accounted for by computer simulation. In particular, the experimental anisotropic diffusion ratio of 1.2 ± 0.1 for water diffusion in the squid giant axon (Table 4.4) falls well within the range of anisotropies calculated for the 60, 30, 25, and 20 nm hexagonal lattice spacings, i.e. 1.05, 1.14, 1.24, and 1.32 respectively (Table 4.5). The "Monte Carlo" technique however, predicts somewhat smaller anisotropies than a previous *analytical* technique (Rorschach 1973), which modelled the diffusion of water in striated muscle by an hexagonal lattice of impenetrable rods representing the myosin-actin lattice. By means of this analytical solution, diffusional anisotropies of 1.1, 1.3, 1.4, and 1.7 are obtained respectively for the 60, 30, 25, and 20 nm lattice spacings. The relatively weak influence on diffusion of the hexagonal array is consistent with the relatively small proportion of the total area occupied by the barriers, namely 3, 10, 15, and 23% of the total area respectively, for the 60, 30, 25, and 20 nm hexagonal lattice spacings. High magnification electron microscopy of transverse sections of nerves shows that only ~ 5-15% of the axoplasmic cross-section seems to be occupied by neurofilaments.

Despite the *apparent* high viscosity of axoplasm, most small ions, such as Na^+ and K^+ , can diffuse through axoplasm nearly as rapidly as in free solution (Rubinson 1979 and references within). The anisotropic diffusion ratio was 1.3 for diffusion of $^{28}\text{Mg}^{2+}$ in the squid giant axon (Baker 1972). The results of ion diffusion in axoplasm correspond well with our measurements of rapid and nearly isotropic diffusion of water in axoplasm. The squid giant axon is composed of ~ 2.5% protein

and 87% water (Brown 1990). Surprisingly, axoplasm behaves as a fairly firm gel, and in fact, extruded axoplasm can emerge as an intact cylinder, the structural support provided by the neurofilamentary lattice (Lasek 1982, Brown 1990). The very small amount of protein is able to give the axoplasm a rigid mechanical strength macroscopically, but is so hydrated that the macromolecular strands are too dispersed in order to inhibit significantly the translational displacement of water or small ions. Hence, the axoplasm is not as viscous, on the molecular scale, as our intuition may lead us to believe, and therefore the diffusion coefficients in pure axoplasm are not reduced to the extent that one might expect.

Investigation of the potential determinants of diffusional anisotropy of water in nerve were precipitated by the somewhat surprising finding (Section 4.1) that water in a nonmyelinated nerve of the garfish, namely, its olfactory nerve, exhibited similar anisotropy to water in its myelinated optic and trigeminal nerves. However, by successively ruling out each of the longitudinal elements of the axons, first, the microtubules and fast axonal transport (Section 4.1) and secondly, the neurofilaments (this section), only the membranes, remain to fulfill the role of the primary determinant of anisotropic water diffusion.

When successive membrane layers wrap to form a myelin sheath around the axon, the source of anisotropic diffusion is not difficult to visualize. In the case of the nonmyelinated olfactory nerve of the garfish however, anisotropic diffusion must depend on successive passages of the water molecules through {or around (Latour 1994a)} the adjacent axonal membranes of the small, densely packed axons, whose diameters ($\sim 0.25 \mu\text{m}$) are significantly less than the RMS displacement of a water molecule ($\sim 5 \mu\text{m}$ for typical NMR diffusion times).

By excluding a dominating role for the neurofilaments, the present work removes the uncertainty left by the garfish studies (Section 4.1) and confirms the role of membranes in producing diffusional anisotropy.

4.3 The Contribution of Local Magnetic-Susceptibility-Induced Gradients to Anisotropic Diffusion⁴

4.3.1 Introduction

The rate and anisotropy of water diffusion in tissue can be related to the immediate structural environment. In addition to the intrinsic viscosity of the cytoplasm, the degree of hindrance to water diffusion is determined by the size, shape, and composition of any physical obstructions, as well as the spacing between those obstructions. For example, it was recently shown that neurofilaments, the thin longitudinally-oriented protein structures found in nerve axoplasm, did not hinder water diffusion to a great extent in the giant axon of the squid (Section 4.2), and as a result it was concluded that the presence of membranes was likely to be the principal factor in hindering water diffusion perpendicular to the long axis of white matter fibers. However, in addition to the physical obstructions to diffusing molecules, local susceptibility-induced gradients⁵ (i.e. background gradients) can alter the diffusion coefficients measured by the typical pulsed-gradient spin-echo (PGSE) pulse sequence in heterogeneous systems (Stejskal 1965, Williams 1978, Karlicek 1980, Zhong 1991b). In fact, it has been suggested that the anisotropic water diffusion coefficients measured in nerve and in white matter might very well be due to the presence of anisotropic background gradients (Lian 1994). Over typical NMR diffusion measurement times (~ 20 - 40 ms), the measured degree of diffusion coefficient anisotropy $\{D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)\}$, is routinely ~ 2 - 4 in white matter and in normal nerve (Moseley 1990a, Le Bihan 1993, Section 4.1) where $D_{\text{NMR}}(\parallel)$ and $D_{\text{NMR}}(\perp)$ are respectively the diffusion coefficients measured parallel and perpendicular to the

⁴ A version of this section has been submitted. C. Beaulieu, P.S. Allen, An evaluation of the effects of local magnetic-susceptibility-induced gradients on anisotropic water diffusion in nerve. *Magn Reson Med* submitted.

C. Beaulieu, P.S. Allen, Testing the effects of local magnetic-susceptibility-induced gradients on diffusional anisotropy in nerve. In Book of Abstracts, 3rd Annual Meeting of the Society of Magnetic Resonance, Nice, p. 356 (1995). Presented as a talk.

⁵ When a material is placed into a magnetic field, the magnetic field in the material can be altered from its value in vacuum. The magnetic field can be increased or decreased depending on whether the sample is paramagnetic or diamagnetic. The magnetic susceptibility relates the net magnetization to the applied field strength. Therefore, gradients (i.e. changes in the field strength as a function of spatial position) can be induced at the interfaces between two compartments with different magnetic susceptibilities.

fiber axis. All or part of any reduction of $D_{\text{NMR}}(\perp)$ relative to $D_{\text{NMR}}(\parallel)$ could be artificially generated in a sample of aligned fibers, since the fibers that are transverse to the static magnetic field (B_0) can give rise to background gradients perpendicular to the fiber axis (but not parallel to it) that make the diffusion coefficients measured perpendicular to the fiber axis appear smaller than their "true" values (Hong 1992, Lian 1994).

As shown previously (Stejskal 1965), background gradients give rise to extra terms in the decay of the echo amplitude arising from a PGSE sequence (see Eqn. 2.45). In particular, the cross-terms between the applied diffusion-sensitizing gradients and the background gradients affect the rate of decay relative to the gradient time integrals and thus affect the diffusion coefficient estimate. In order to determine the magnitude of the background gradient effect on the anisotropy of diffusion coefficient measurements, measurements must be made with and without the cross-term effects. A general technique for eliminating the cross-term effects on D_{NMR} (Hong 1992) is by means of a spin-echo technique incorporating bipolar gradient pulse pairs which are antisymmetrically polarized about the π radiofrequency (rf) pulse (BGP in the nomenclature of Hong 1992). In contrast, bipolar gradient pulse pairs in which the second pair is reversed (BGPR), i.e. which are symmetrically polarized about the π rf pulse in a spin-echo sequence, do not eliminate the effect of the background gradients on D_{NMR} (Hong 1992) (see Fig. 3.13 for a block diagram of these diffusion pulse sequences). Diffusion measurements using alternately BGP and BGPR pulse sequences with identical timings can therefore isolate the effect of background gradients since the diffusion times, and hence, the influence of physical restrictions will be equivalent for both pulse sequences (Trudeau 1994).

An alternative way to observe the effects of background gradients is to change the magnitude of the background gradients significantly. In principle, in perfectly aligned fibers, the susceptibility-induced gradients can be eliminated by longitudinally orienting the fibers along B_0 and can be maximized by making the fibers and B_0 normal to each other. The purpose of this section is to report an evaluation of the influence of background gradients on the measurement of the orthogonal diffusion coefficients of water in excised nerve fibers at 2.35 T. Diffusion measurements (PGSE and BGP) with the nerve axis aligned longitudinally along B_0 were compared to measurements (PGSE, BGP, and BGPR) obtained when the nerve fiber orientation

was transverse. In particular, the olfactory, trigeminal, and optic nerves of the garfish and the sciatic nerve of the frog were used in these investigations.

4.3.2 Results and Discussion

In view of the possibility of a null finding, it was important to establish first the validity of the experimental technique that was used in our measurements of diffusion. Table 4.6 lists the diffusion coefficient measurements obtained for water protons, first in distilled water and secondly, in order to provide a comparison with the previous work of Hong and Dixon (1992) and Lian et al. (1994), in apple flesh. In distilled water, all measurements of D_{NMR} agree to within the limits of measurement error ($\sim 3\%$), indicating an independence of measuring sequence. The mean D_{NMR} magnitude from all measurements is within 4% of that previously reported at 19 °C (Mills 1973). Moreover, the anisotropy measurements are all within 2% of unity, indicating excellent isotropy. The situation in apple flesh is different from distilled water, but nevertheless in agreement with previous independent data (Lian 1994, Hong 1992). For example, a clear sequence dependence exists showing the same trend as reported in Hong and Dixon (1992) in the spatially averaged mean D_{NMR} , namely, $D_{\text{NMR}}(\text{BGP}) > D_{\text{NMR}}(\text{BGPR}) > D_{\text{NMR}}(\text{PGSE})$. Our mean $D_{\text{NMR}}(\text{BGP})$ value devoid of any cross-term effects agrees with the corresponding measurements of Hong and Dixon (1992) and Lian et al. (1994) within the limits of our error (no error was quoted in either of these publications). The more substantial reduction in mean D_{NMR} between the BGP and PGSE sequences, which is only half that of Hong and Dixon (1992), i.e. $\sim 40\%$ compared with $\sim 87\%$, could be due to our smaller field strength (i.e. 2.35 T instead of Hong and Dixon's 4.7 T) and/or to the maturity of our apple (Lian 1994). We also report an apparent asymmetry of about 10% in D_{NMR} for the apple, with reference to the specimen tube axis, when that axis is transverse to B_0 . Such asymmetries were averaged, but not quoted, in Hong and Dixon (1992).

Table 4.6 Measurements of the diffusion coefficients (D_{NMR}) and their anisotropy relative to the sample axis, for water protons in distilled water and in apple flesh, at two different orientations of the sample axis relative to the static magnetic field ($B_0 = 2.35 \text{ T}$) ^{a, b}.

Sample	Variable	Sample axis transverse to B_0			Sample axis longitudinal to B_0	
		PGSE	BGP	BGPR	PGSE	BGP
Distilled water ^{c, d}	$D_{\text{NMR}}(\parallel)$ ^e	2.05	2.00	2.05	1.96	1.98
	$D_{\text{NMR}}(\perp)$ ^e	2.02	2.00	2.02	2.00	2.00
	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$	1.0	1.0	1.0	1.0	1.0
Apple	$D_{\text{NMR}}(\parallel)$ ^e	1.14 ± 0.08 ^f	1.62 ± 0.07 ^g	1.41 ± 0.05 ^g	—	—
	$D_{\text{NMR}}(\perp)$ ^e	1.04 ± 0.06	1.49 ± 0.22	1.24 ± 0.07	—	—
	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$	$1.1 \pm <0.1$	1.1 ± 0.2	1.1 ± 0.1	—	—
	Mean D_{NMR} ^e	1.09 ± 0.08	1.55 ± 0.17	1.33 ± 0.11	—	—

^a Measurements made at room temperature ($\sim 19^\circ\text{C}$).

^b The gradient pulse timings were $\delta = 6 \text{ ms}$ and $\Delta = 30 \text{ ms}$ in the diffusion pulse sequences.

^c $D(\text{distilled H}_2\text{O})$ is reported to be $1.94 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 19°C (Mills 1973).

^d For a single measurement the r^2 of the best fit was ≥ 0.999 . For repeated measurements, typical errors were $\sim 0.05 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$.

^e D_{NMR} values are quoted in units of $\text{cm}^2 \text{ s}^{-1} \times 10^{-5}$.

^f Averaged over 8 measurements, the errors quoted are the standard deviations.

^g Averaged over 4 measurements, the errors quoted are the standard deviations.

The ability, with excised nerve samples, to align the axonal channels relative to B_0 provides a controlled means of substantially modulating certain local background gradients, maximizing them when the axonal membrane surfaces are transverse to B_0 and minimizing them when the axons are longitudinally oriented along B_0 . Such gradients will be perpendicular to the axonal channels and should have their greatest effect on NMR measurements of diffusion across that channel, i.e. on $D_{\text{NMR}}(\perp)$, thereby making $D_{\text{NMR}}(\perp)$ dependent on orientation with respect to B_0 when measured with the PGSE sequence and maximizing the sequence dependence at the transverse

orientation. The data for the four types of excised nerve are illustrated in Fig. 4.9. Unlike the apple or distilled water, excised nerve, as reported previously (Section 4.1), clearly exhibits anisotropic diffusion of its water protons, diffusion parallel to the axonal axis being much easier than that perpendicular to it. Taking an overall view of all the nerves, the important characteristic of the diffusion coefficient data is the singular consistency, within the limits of error, of $D_{\text{NMR}}(\perp)$, independent of both measuring sequence and axonal orientation relative to B_0 . This observation is strongly suggestive that in nerve, and therefore presumably in white matter, at 2.35 T (and therefore at lower, more clinically relevant field strengths) the local gradients do not contribute measurably to $D_{\text{NMR}}(\perp)$ and consequently to the diffusion coefficient anisotropy.

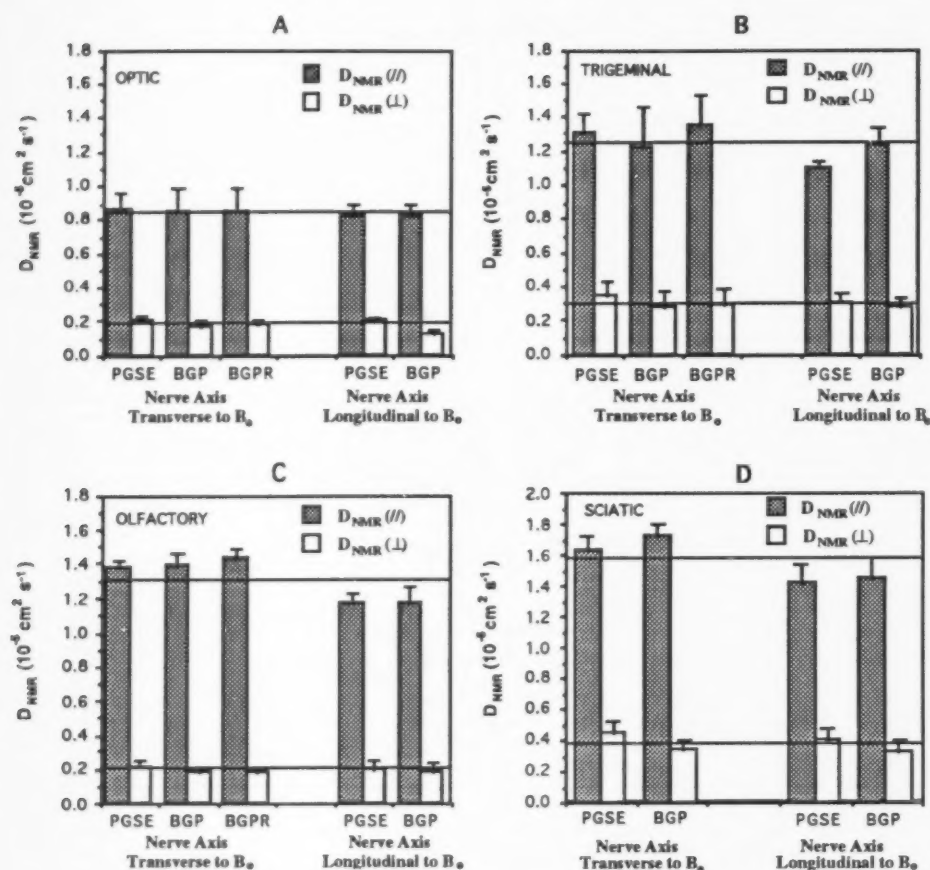


Figure 4.9 Measurements of water proton diffusion coefficients parallel ($//$) and perpendicular ($\perp$) to the length of nerve fibers as obtained with the PGSE, BGP, and BGPR sequences. The fiber alignment was either transverse or longitudinal relative to the static magnetic field ($B_0 = 2.35 \text{ T}$). The nerve fibers evaluated were the optic (A), trigeminal (B), and olfactory (C) nerves of the garfish and the sciatic nerve of the frog (D). The two horizontal lines in each plot represent the mean $D_{\text{NMR}}(//)$ and mean $D_{\text{NMR}}(\perp)$ values over all of the measurements performed for each particular nerve.

Although the above conclusion about the influence of local gradients is drawn from the overall conformity of data from four types of nerve, certain other features of that data shown in Fig. 4.9 are deserving of comment. For example, in three cases the mean $D_{\text{NMR}}(//)$ that is measured under longitudinal alignment along B_0 is less ($\leq 15\%$)

than the mean $D_{\text{NMR}}(\parallel)$ measured under transverse alignment. Such an orientational dependence of $D_{\text{NMR}}(\parallel)$ is not anticipated from the B_0 induced background gradients and is not fully understood. Imperfections in the horizontal alignment of the longer, finer nerves, as compared with the short optic nerve which shows no orientational dependence, would invalidate the assumed correspondence between the horizontal D_{NMR} and $D_{\text{NMR}}(\parallel)$, thereby reducing the former. However, such imperfections would not be expected to produce a change of 15%. A second comment is in order with reference to the mean D_{NMR} values, averaged over all of the data presented in Fig. 4.9, and which are listed in Table 4.7. The anisotropies quoted in Table 4.7 are greater than those previously reported by us (Table 4.2). The previous data were acquired from a batch of small, immature garfish, whereas the data presented here were acquired from mature garfish whose weights were typically an order of magnitude greater than their immature predecessors.

Table 4.7 A summary of the mean water proton diffusion coefficients parallel and perpendicular to the nerve axis ^{a, b}.

Sample	$D_{\text{NMR}}(\parallel)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$
Optic (N=30)	0.84 ± 0.10	0.18 ± 0.04	4.9 ± 1.3
Trigeminal (N=30)	1.22 ± 0.19	0.31 ± 0.07	4.3 ± 1.5
Olfactory (N=30)	1.29 ± 0.15	0.19 ± 0.04	6.9 ± 1.5
Sciatic (N=40)	1.57 ± 0.17	0.36 ± 0.08	4.6 ± 1.1

^a Measurements were made at room temperature ($\sim 19^\circ \text{C}$).

^b The means were derived from all the measurements presented in Fig. 4.9. The errors quoted are the standard deviations.

The null finding in regard to the background gradient influence on $D_{\text{NMR}}(\perp)$ in excised nerve serves to consolidate the conclusion of some earlier work (Sections 4.1 and 4.2), namely, that multiple membrane encounters are the principal mechanism of diffusional anisotropy in nerve, irrespective of whether those membranes are wrapped into a myelin sheath or exist as single membranes surrounding very fine, densely packed, nonmyelinated axons. The restrictive influence of membranes in the examples evaluated here was demonstrated by substantially shortening the diffusion time, t_d ,

from 28 ms to 2 ms. By using shorter diffusion times, the diffusing water has less time to interact with the restrictive surroundings. If diffusion is restricted the diffusion coefficients will increase as t_d is decreased, whereas unrestricted diffusion would result in no change of D_{NMR} . The demonstration was carried out using the background gradient insensitive sequence, BGP, and it was found (see Table 4.8) that although the t_d shortening had minimal effect for distilled water and apple, it produced more than a two-fold increase in $D_{\text{NMR}}(\perp)$ for the frog sciatic nerve, which was accompanied by an equivalent decrease in the derived anisotropy. Since there are relatively few barriers to diffusion along the length of the nerve, the change produced in $D_{\text{NMR}}(\parallel)$ by t_d shortening did not exceed the limits of error.

Table 4.8 An illustration of the change in water proton diffusion coefficients and their anisotropy due to a shortening of the diffusion time, t_d , in distilled water, apple, and the sciatic nerve of the frog ^{a, b}.

Sample	Diffusion time (t_d)	$D_{\text{NMR}}(\parallel)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$
Distilled water ^c	28 ms	2.04 ± 0.06	1.99 ± 0.03	$1.0 \pm <0.1$
	2 ms	2.07 ± 0.01	2.03 ± 0.02	$1.0 \pm <0.1$
Apple ^d	28 ms	1.62 ± 0.07	1.49 ± 0.22	1.1 ± 0.2
	2 ms	1.50 ± 0.07	1.45 ± 0.05	$1.0 \pm <0.1$
Sciatic nerve	28 ms	1.73 ± 0.07 ^e	0.34 ± 0.05	5.3 ± 0.8
	2 ms	1.65 ± 0.10 ^f	0.81 ± 0.07	2.1 ± 0.1

^a Measurements were made at room temperature ($\sim 19^\circ\text{C}$).

^b For water and apple, $\parallel$ and $\perp$ refer to the orientation with respect to the sample axis. For the sciatic nerve, $\parallel$ and $\perp$ refer to the orientation with respect to the axonal axis. For all samples, the long axis is perpendicular to B_0 .

^c Averaged over 3 samples, the errors quoted are the standard deviations.

^d Averaged over 4 samples, the errors quoted are the standard deviations.

^e Averaged over 11 samples, the errors quoted are the standard deviations.

^f Averaged over 8 samples, the errors quoted are the standard deviations.

What is even more encouraging is the remarkable agreement between the experimental frog sciatic nerve data of Table 4.8 and the model simulation results

illustrated in Fig. 3 of the paper by Szafer et al. (1995). Axonal diameters of the frog sciatic nerve range from 4 to 10 μm and, for a model system of very similar dimensions, the simulations of Szafer et al. predict an increase in $D_{\text{NMR}}(\perp)$ from $\sim 0.35 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ to $\sim 0.7 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ as a result of t_d shortening from 28 ms to 2 ms. The excellent agreement is thus both in the absolute magnitudes and in the relative change.

In the literature, there have been several reports on the testing of restricted diffusion in white matter by varying the diffusion time (Moonen 1991, Le Bihan 1993, Gates 1994, Horsfield 1994). These imaging studies have used diffusion times in the range of 20 - 80 ms (Le Bihan 1993), 50 ms - 2 s (Moonen 1991), 40 ms - 1 s (Gates 1994), and 40 - 600 ms (Horsfield 1994). Two of these studies reported a decrease in the magnitude of the diffusion coefficients as t_d was increased ($D_{\text{NMR}}(\perp)$ in Gates 1994, $D_{\text{NMR}}(\parallel)$ and $D_{\text{NMR}}(\perp)$ in Horsfield 1994) in contrast to the other two studies that reported no such dependence (Moonen 1991, Le Bihan 1993). The latter two studies concluded from this independence of D_{NMR} on t_d that cell membranes only impose minor restrictions to water diffusion. However, all of these studies are limited in that their shortest diffusion times are quite large. Others have shown that changes in the measured diffusion properties of water in brain can be observed if one varies t_d over short enough times, such as 3.4-6 ms (Niendorf 1994). According to Szafer et al. (1995), $D_{\text{NMR}}(\perp)$ in model systems with diameters similar to axons should change markedly at short diffusion times (most of the drop occurs in the first 10 ms) and then level off at diffusion times greater than 20 ms.

4.3.3 Conclusion

The singular consistency of $D_{\text{NMR}}(\perp)$ measurements in nerve, independent of both measuring sequence and axonal orientation relative to B_0 , is not consistent with the hypothesis of a measurable role for background gradients in the determination of water proton diffusion coefficients in nerve at $B_0 \leq 2.35 \text{ T}$. This null finding, together with the significant increase in $D_{\text{NMR}}(\perp)$ at shorter diffusion times in the frog sciatic nerve, consolidates the previous conclusion (Section 4.2) that multiple membrane encounters are the principal mechanism for diffusion coefficient anisotropy in nerve and, presumably, in white matter.

CHAPTER 5

Diffusion Characteristics of T_2 Discriminated Water Pools in Nerve ⁶

5.1 Introduction

Transverse relaxation of water protons is often multi-exponential in the white matter of brain, in spinal cord, and in nerve, both in-vitro and in-vivo. The transverse relaxation decay curves obtained for these tissues are generally fitted with three components (sometimes two), namely, a long transverse relaxation time (T_2) component $\sim 200 - 600$ ms, an intermediate T_2 component $\sim 70 - 200$ ms, and a short T_2 component $\sim 10 - 50$ ms (see Table 2.1). These multiple T_2 components are frequently interpreted in terms of distinct water pools within the heterogeneous tissue. For example, the long, intermediate, and short T_2 components observed in the aforementioned nervous tissues are usually assigned to water in the inter-axonal (i.e. connective tissue space in the garfish nerves), axoplasmic, and myelin domains, respectively. There is considerable evidence supporting the hypothesis of water compartmentalization as the source of the multi-exponentiality in the transverse relaxation decay curves of water in ordered axonal systems (as discussed in Section 2.2.1).

In addition to transverse relaxation times, nuclear magnetic resonance measurements of the self-diffusion coefficient (D_{NMR}) can further characterize tissue water, the degree and direction of diffusion being an indicator of the tissue microstructure. For example, water diffusion, as measured by NMR, is anisotropic in the white matter of cat (Moseley 1990a) and human brain (Chenevert 1990), in vivo, and in excised garfish nerve (Section 4.1). Namely, diffusion parallel to the length of the axons is more efficient than perpendicular to it. The compartmentalized nature of nervous tissue complicates the interpretation of the observed diffusion coefficients. Routine spin-echo measurements of water diffusion in nervous tissue measure an apparent diffusion coefficient that is a function of diffusion, T_2 , and proton populations of water in the various compartments of tissue. Measuring the diffusive

⁶ A version of this work was presented at a conference. C. Beaulieu, P.S. Allen, The diffusion characteristics of T_2 discriminated water pools in nerves. *In* Book of Abstracts, 12th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 1626 (1993).

capabilities of water in the individual compartments would improve our understanding and characterization of the properties of water in tissue, the ultimate goal being to improve the specificity of MRI in the diagnosis of various nervous system pathologies.

In order to measure the diffusive properties of only one of the compartments (e.g. the inter-axonal space), we need to attenuate the signal from the other compartments (e.g. myelin, axonal space). In particular, the diversity in the transverse relaxation times of the multiple components can be used to attenuate the shorter T_2 components and preserve the long T_2 component. Shorter T_2 components can be reduced in amplitude (and eventually eliminated) from the magnetization vector by moving to longer times along the transverse relaxation decay curve. Therefore, the diffusive properties of the longer T_2 component(s) can be measured preferentially by incorporating into the diffusion measuring pulse sequence a Carr-Purcell-Meiboom-Gill (CPMG) sub-sequence (van Dusschoten 1992) (see Fig. 3.14) using a sufficient number of π radiofrequency pulses to extend the decay.

The purpose of this chapter is to report measurements of water diffusion at incremented CPMG spin-echo times in the nonmyelinated olfactory nerve, and the myelinated trigeminal and optic nerves of the garfish, which are used as white matter models.

5.2 Results

To verify the accuracy and isotropy of D_{NMR} over a range of spin-echo times, NMR self-diffusion measurements of distilled water were performed (Fig. 5.1A). As expected, diffusion was isotropic (i.e. $D_{\text{NMR}}(\parallel) = D_{\text{NMR}}(\perp)$) and the mean D_{NMR} value (see Table 5.1) was within 5% of the expected self-diffusion coefficient of pure water at 20°C (Mills 1973). In all three types of excised garfish nerve, the magnitude and anisotropy of D_{NMR} did not change markedly as the spin-echo time (TE) was incremented from 70 to 450 ms (Fig. 5.1B-D). The mean $D_{\text{NMR}}(\parallel)$, $D_{\text{NMR}}(\perp)$, and anisotropic diffusion ratios (i.e. $D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)$) over the full range of spin-echo times investigated for pure water, and water in the olfactory, trigeminal, and optic nerves of the garfish are shown in Table 5.1. Figure 5.2 illustrates the loss of

transverse magnetization of nerve water protons as the spin-echo time was incremented in a CPMG-diffusion pulse sequence (Fig. 3.14) with no applied gradients (i.e. $G_1 = 0$). Approximately 50 - 65 % of the initial proton magnetization of nerve water persisted at $TE = 70$ ms whereas only ~ 3 - 10 % of the original signal survived at $TE = 450$ ms (Fig. 5.2). The magnitude of the residual signal from nerve water at these echo times is in accordance with that expected from the component T_2 values and their respective populations (as determined from Table 2.1).

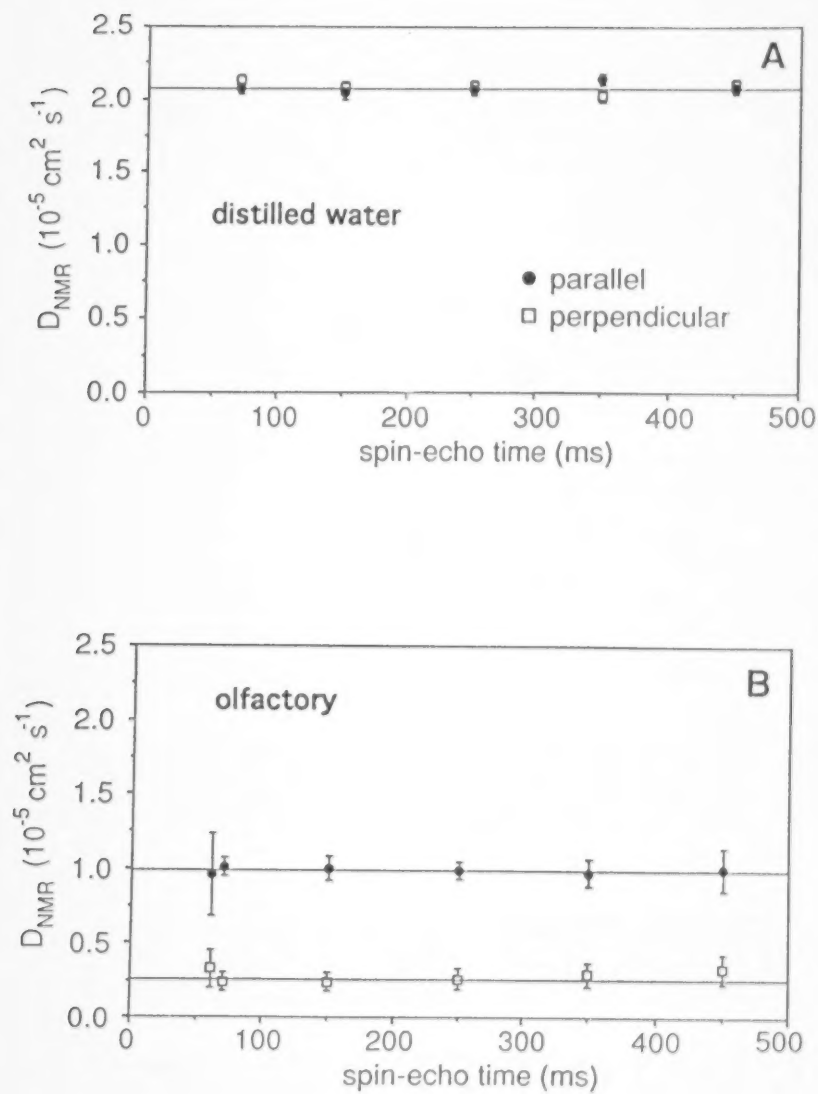


Figure 5.1 Figure caption on next page.

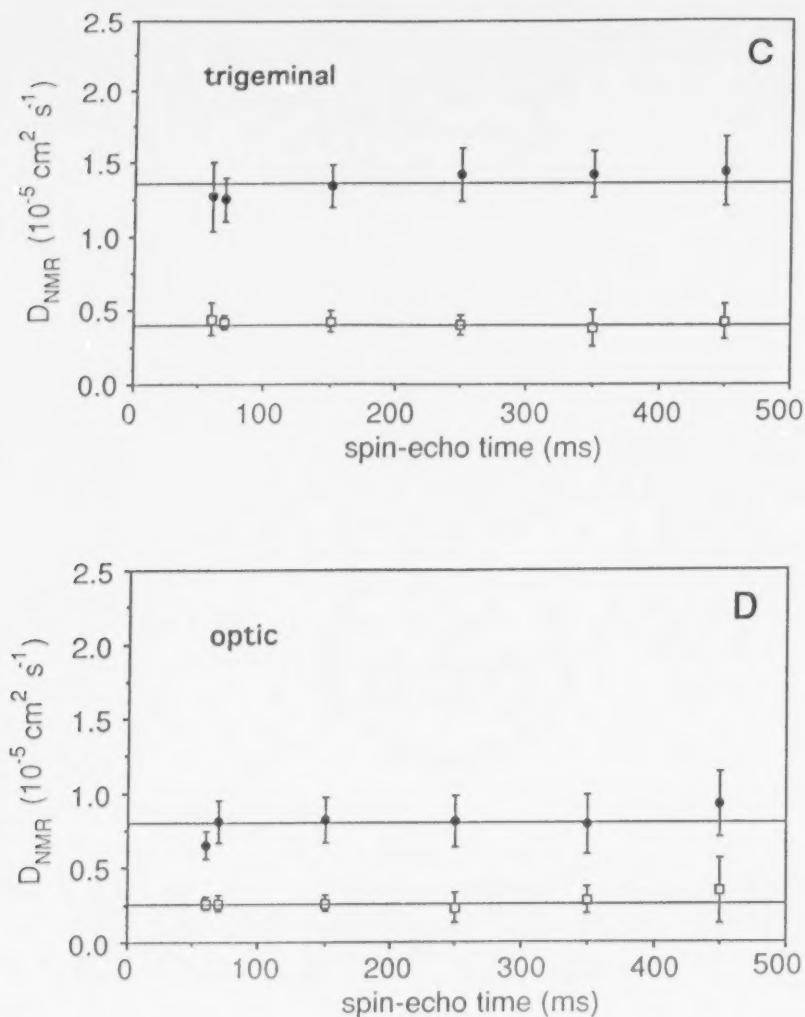


Figure 5.1 Mean diffusion coefficients, D_{NMR} , of water measured parallel and perpendicular to the long axis of a distilled water sample (A), and freshly excised olfactory (B), trigeminal (C), and optic (D) nerves from six garfish as the spin-echo time was varied (70, 150, 250, 350, 450 ms). These D_{NMR} values are compared to those of eleven garfish which were previously acquired using a routine spin-echo diffusion sequence with a spin-echo time of 60 ms (Table 4.2). The vertical error bars correspond to the 95% confidence intervals. The horizontal bars represent the mean D_{NMR} values over all of the spin-echo times (70-450 ms). Notice the isotropy of diffusion for the distilled water phantom (A) in contrast to the anisotropy of water diffusion observed in the nerves (B, C, D).

Table 5.1 NMR measurements of water diffusion in distilled water, and the olfactory, trigeminal, and optic nerves of the garfish at spin-echo times ranging from 70 to 450 ms ^{a, b}.

Sample	$D_{\text{NMR}}(\parallel)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$D_{\text{NMR}}(\perp)$ ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$
Distilled water	2.08 ± 0.05	2.08 ± 0.05	1.0 ± 0.1
Olfactory nerve	1.00 ± 0.04	0.27 ± 0.05	3.7 ± 0.5
Trigeminal nerve	1.37 ± 0.09	0.41 ± 0.04	3.4 ± 0.3
Optic nerve	0.81 ± 0.05	0.27 ± 0.04	3.1 ± 0.3

^a Measurements were made at room temperature ($20 \pm 1^\circ \text{C}$).

^b The mean $\pm$ 95% confidence interval are given for the results at all of the spin-echo times (70, 150, 250, 350, and 450 ms) that were used in the CPMG-diffusion pulse sequence.

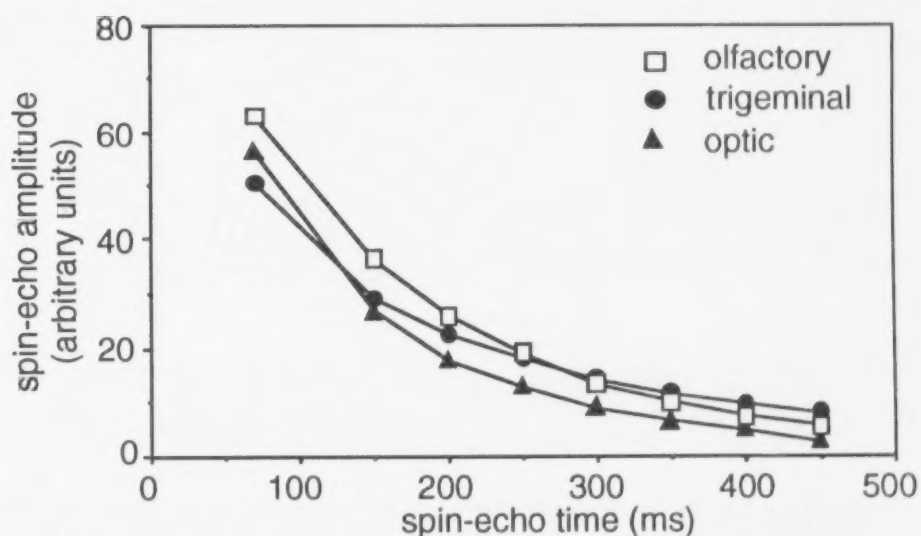


Figure 5.2 An example of the decay of water proton transverse magnetization in excised garfish nerves as the spin-echo time (TE) in the CPMG-diffusion pulse sequence was augmented. The echoes are obtained at various spin-echo times using no diffusion-sensitizing gradients. The amplitudes are normalized to an extrapolated signal amplitude of 100 at TE = 0 ms.

5.3 Discussion

We have shown that D_{NMR} of water in three different types of excised garfish nerve is independent of the spin-echo time (at least over the range 70 - 450 ms) in a CPMG-diffusion pulse sequence. In other words, the decreased contribution of shorter T_2 components to the net transverse magnetization at longer spin-echo times has not altered the measured properties of diffusion. Moreover, the magnitudes and anisotropies of D_{NMR} in the garfish nerves are similar to human in-vivo values (Table 2.2), indicating that garfish nerves are a suitable model for water diffusion in the human central nervous system (white matter in particular). Based on the component T_2 values and their respective populations (Table 2.1), we expect that ~ 30 - 40 % and ~ 80 - 90 % of the total nerve water signal at $TE = 70$ ms and 450 ms, respectively, are due to water from the inter-axonal space. Hence, most of the magnetization at $TE = 450$ ms should originate from the long T_2 component, and our measurements indicate that the diffusive abilities of this component are hindered and anisotropic. Diffusional anisotropy seen in long spin-echo time (~ 240 ms) weighted images of human white matter, in-vivo, (Hajnal 1992b, Oatridge 1993) support our experimental results on the garfish nerves.

If we hypothesize that the long T_2 component seen in nerves and white matter corresponds to the inter-axonal space water (evidence for this hypothesis is discussed in Section 2.2.1), then the mobility of the water in the inter-axonal space is hindered and anisotropic. Diffusion measurements of water in freshly excised squid giant axons have shown that the majority of the anisotropy and reduced D_{NMR} of water in nerves is due to the presence of membranes (Section 4.2). Averaged over all of the garfish nerves, the one-dimensional root-mean-square displacements ($\lambda_1 = (2Dt_d)^{1/2}$) for a diffusion time, t_d , of 28 ms are $7.3 \pm 1.3 \mu\text{m}$ parallel and $4.4 \pm 0.6 \mu\text{m}$ perpendicular to the long axis of the nerves, whereas λ_1 for pure water would be ~ 11 μm . Hence, over the experimental diffusion time, in our case ~ 28 ms, the inter-axonal water molecules moving perpendicular to the long axis of the fibers would experience many membranous barriers since the observed $\lambda_1(\perp)$ is larger than typical separations between individual barriers (~ 1 μm between adjacent myelin sheaths in the myelinated nerves and ~ 0.5 - 2 μm between Schwann cell domains of axons in the nonmyelinated nerve). Thus the mobility of the inter-axonal water perpendicular to the

nerve axis is hindered to a greater extent than that parallel to the nerve axis, resulting in anisotropic diffusion.

Similar anisotropy for the inter-axonal water would be expected for adult white matter in which the small amount of inter-axonal water (Nicholson 1992) (~ 15 - 20%) is distributed thinly between numerous packed axons (including their myelin sheaths if present). Previously, others have concluded that the mechanism of molecular hindrance is related to the dense packing of membranes, and hence, the brain intracellular and inter-axonal microenvironments exhibit similar tortuosities and should impede water diffusion to a similar extent (Nicholson 1993). Our results agree with their conclusion. If the diffusive environments of the intracellular and inter-axonal spaces are similar, one consequence would be that the nonlinear behaviour of the diffusion curves observed in garfish nerves at high b values (Fig. 4.1) is likely due to restricted diffusion, and not due to multiple water compartments with different diffusional characteristics.

Diffusion coefficients are poorly (or not) correlated with T_2 values of water in various pathologies of nervous tissue (Goldberg 1992, Christiansen 1993). It is generally assumed that a compartment with a large amount of macromolecular structure should have a short T_2 constant and a small D value. Conversely, the "lack" of macromolecules in the inter-axonal space is often used to justify the *assumption* of rapid inter-axonal diffusion. Agarose gels are one example in which there is a significant reduction in T_2 with almost no change in diffusion (Table 5.2). This reduction in T_2 , relative to T_1 , has been attributed to a small fraction of water that is bound and efficiently relaxed by the macromolecules, the remainder of the water being relatively free (Woessner 1970). Hence, the mechanism responsible for decreased T_2 's does not decrease D_{NMR} markedly, and therefore, a general correlation between T_2 and D_{NMR} in biological systems is not expected. Furthermore, the *intrinsic* (i.e. no membrane interactions) diffusive mobility of water in both the intra- and inter-axonal compartments of nerve (and presumably white matter) could be similar and quite rapid, despite their apparent differences in transverse relaxation. Over typical NMR diffusion measurement times, the membranes in nerve could hinder both intra- and inter-axonal (perpendicular) water diffusion to the same extent, and hence could generate similar degrees of anisotropy for these two compartments of water in nerve.

Table 5.2 Diffusion coefficients (D_{NMR}), transverse relaxation times (T_2), and longitudinal relaxation times (T_1) of distilled water, and water in 1.5% and 3% agarose gels ^a.

Samples	$D_{\text{NMR}}(\parallel)$	$D_{\text{NMR}}(\perp)$	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$	T_1 (ms)	T_2 (ms)	$\frac{T_1}{T_2}$
	$(10^{-5} \text{ cm}^2 \text{ s}^{-1})$					
Distilled water	2.08	2.08	1.0	2700	2000	1.4
1.5% agar gel	1.99	1.94	1.0	2200	100	22
3% agar gel	1.96	1.95	1.0	1900	53	37

^a Measurements made at room temperature ($20 \pm 1^\circ \text{C}$).

Recently, there has been great interest in diffusion-weighted magnetic resonance imaging (MRI) since it can detect regions of cerebral ischemia sooner than the usual T_2 -weighted MRI (Moseley 1990b) (discussed in Section 2.2.2). In particular, the region of ischemic damage demonstrates lower D_{NMR} values ($\sim 50\%$) than that found in normal cerebral tissue (Knight 1991). There is still considerable debate on the source of the decreased diffusion coefficient in the stroke-affected region. One hypothesis is that there is a water shift from a *freely diffusing* inter-axonal environment to a more restricted intracellular environment in the ischemic region. In order to explain the observed reduction of D_{NMR} for a region of acute cerebral ischemia in a rat, the $D(\text{interstitial})$ was assumed to be ~ 23 times greater than $D(\text{intracellular})$ (Beneviste 1992). In another study of an acute stroke model in cat brain, $D(\text{inter-axonal})$ was required to be ~ 7 times greater than $D(\text{intracellular})$ in order to calculate the observed (bicomponent averages in this model) D_{NMR} values for healthy and infarcted tissue (van Gelderen 1994b). Most of the reported reductions in diffusion with the onset of ischemia have been for water in the grey matter. However, decreases in an average D_{NMR} value, with no changes in anisotropy, have also been observed in ischemic white matter (van Gelderen 1994b). Others have also observed decreased diffusion of water in ischemic white matter (Dardzinski 1993). As shown in this chapter, the interstitial water (i.e. long T_2 component) likely has diffusive properties which are not much different from the intracellular (axoplasmic) space. Moreover, measurements of water diffusion in squid giant axons (Section 4.2) have

shown that water diffusion in axoplasm, with little interference from the axonal membrane, is only slightly less than that of pure water and is weakly anisotropic. One cannot, therefore, assume a much smaller intrinsic D value for water in the intra-axonal space than in the inter-axonal space of white matter. An extension of this argument to grey matter is not likely valid, since it appears that water in the cytoplasm of neuronal cell bodies may have a much lower intrinsic diffusion coefficient (Schoeninger 1994) than in axoplasm.

5.4 Conclusion

The magnitude and anisotropy of water diffusion in three different types of freshly excised garfish nerve are invariant to increments in the length of the preparatory CPMG sequence (70 - 450 ms) in a CPMG-diffusion pulse sequence. Based on the hypothesis that the long T_2 component observed in nerve corresponds to the water in the inter-axonal compartment, then water diffusion in the inter-axonal space of nerve, over typical NMR measurement times, is hindered and anisotropic.

CHAPTER 6

Changes in Water Diffusion due to Wallerian Degeneration in Peripheral Nerve ⁷

6.1 Introduction

The importance of membranes in generating anisotropic water diffusion in ordered axonal systems such as nerve and white matter has been proposed by many groups (Thomsen 1987, Moseley 1990a, Chenevert 1990, Turner 1990b, Doran 1990, Douek 1991, Sakuma 1991). This postulate has been justified for normal nerve by the following two experimental demonstrations. The first was that large single axons which are devoid of multiple membranes show very little anisotropy (Section 4.2). The second was that susceptibility-difference induced transverse axonal field gradients are not responsible for the marked anisotropy observed in axonal systems that do contain multiple membranes (Section 4.3). However, the association of a specific loss of membranes in injured nerve with a specific change in the degree of diffusion coefficient anisotropy, falls short of that needed to establish the firm correspondence which is the essential precursor to the estimation of specific microstructural change from water diffusion measurements. For example, anisotropy of the diffusion coefficients parallel and perpendicular to ordered axons (i.e. $D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)$) has been found to decrease from the 2.4 observed in normal individuals to 1.5 in lesions of the pyramidal tract (Segawa 1993). However, no direct comparisons between changes in anisotropy and histopathology were performed in this experiment. Reductions in anisotropy were also observed in the lateral and dorsal white matter of rat spinal cords injured by a weight drop (Ford 1994). Specifically, the normal anisotropy of 5 - 6 was reduced to 0.9 - 1.1 in the injured (abnormal-appearing) white matter of the spinal cord (formalin fixed) of rat. Unfortunately, the effect of fixation on the diffusion characteristics of water in these samples of spinal cord is not known. In addition, marked decreases in anisotropy have been observed in garfish nerves which have degenerated in-vitro (Table 4.2). However, the pathologic process whereby nerves degenerate in-vitro may not be expected to correspond to nerves

⁷ A version of this chapter has been presented at a conference. C. Beaulieu, M.D. Does, R.E. Snyder, P.S. Allen, Water diffusion in degenerated sciatic nerve of the frog. *In* Book of Abstracts, 3rd Annual Meeting of the Society of Magnetic Resonance, Nice, p. 1319 (1995). Presented as a poster.

degenerating in-vivo. Experiments are therefore required where the correspondence to in-vivo degradation conditions are much closer and where the degradation can be monitored by independent histological means.

A convenient and universally accepted model for studying nerve degeneration in-vivo is the portion of peripheral nerve that is distal to an inflicted nerve injury. For example, crushing a peripheral nerve, which interrupts axonal continuity with the cell body, causes Wallerian degeneration but also allows subsequent regeneration to occur distal to the crush. In contrast, occluding the nerve at the injury site with a tie prevents regeneration. Wallerian degeneration is characterized by structural changes such as axonal dissolution, followed by contraction and fragmentation of myelin, and finally disintegration and removal of myelin and other debris by Schwann cells and macrophages (Schroder 1975). This degenerative process can occur at different rates depending on the temperature and species of interest. For example, Wallerian degeneration is normally delayed in onset and proceeds more slowly in cold-blooded animals (such as amphibians) than it does in mammals (Sunderland 1968). To determine the correspondence between any changes in the water proton diffusion coefficients and nerve degeneration in-vivo, we have chosen to evaluate the effects resulting from both crush- and tie-injuries in a peripheral nerve, namely, the sciatic nerve of the frog. Changes in the measured diffusion coefficients are compared with axonal and myelin breakdown and the change in spacing of the structural features observed in light and electron micrographs of the damaged sciatic nerves.

6.2 Results and Discussion

Mean $D_{\text{NMR}}(\parallel)$, $D_{\text{NMR}}(\perp)$, and anisotropic diffusion ratios $\{D_{\text{NMR}}(\parallel) / D_{\text{NMR}}(\perp)\}$ for water in nineteen normal, five tie-injured, and six crush-injured sciatic nerves of the frog are given in Table 6.1. The sciatic nerves that underwent in-vivo Wallerian degeneration all showed marked decreases in $D_{\text{NMR}}(\parallel)$, marked increases in $D_{\text{NMR}}(\perp)$, and consequently a much reduced anisotropy in comparison to uninjured nerve (Table 6.1). These alterations in the diffusion coefficients are similar to those observed in a rat spinal cord injury model (Ford 1994); however, in the present case, the nerves have not been fixed prior to the diffusion measurements. Changes in water mobility can be rationalized by noting the structural differences (of the membrane components

in particular) between normal and degenerated nerve from the light and electron micrographs (Figs. 6.1 - 6.3).

Table 6.1 NMR measurements of diffusion coefficients of water in normal, crush injured, and tie injured sciatic nerves from the frog ^{a, b}.

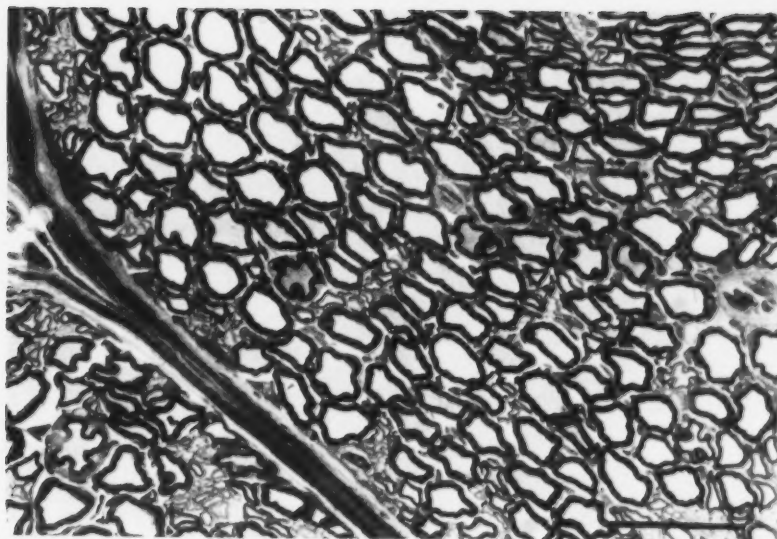
State of Sciatic Nerve	$D_{\text{NMR}}(\parallel)$	$D_{\text{NMR}}(\perp)$	$\frac{D_{\text{NMR}}(\parallel)}{D_{\text{NMR}}(\perp)}$
	($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	
Normal (N=19)	1.63 ± 0.04	0.44 ± 0.04	3.9 ± 0.4
Tie injured (N=5)	1.19 ± 0.04	0.72 ± 0.04	1.7 ± 0.1
Crush injured (N=6)	1.29 ± 0.10	0.59 ± 0.14	2.3 ± 0.4

^a Measurements were made at room temperature ($19 \pm 1^\circ \text{C}$).

^b The mean $\pm$ 95% confidence interval for measurements on N samples.

In normal sciatic nerve of the frog, typical axonal diameters (not including the myelin sheath) range from 4 to 10 μm , with a small minority of axons being much larger (15 - 20 μm). The thickness of the myelin sheath about the axons can range from $\sim 0.5 \mu\text{m}$ for smaller axons ($< 3 \mu\text{m}$ in diameter) to 1.5 - 2 μm for larger axons. The extracellular separations between the exterior of the myelin sheaths tend to range from 0.5 to 2 μm . The normal axons have good "circular" shapes and are packed together quite regularly (Fig. 6.1a). Neurofilaments and mitochondria are visible within the axoplasm (Fig. 6.1b), indicative of a structurally sound nerve. The myelin is continuous, for the most part, with very few fractures and degradations in its lamellar structure. Since, over a diffusion time of 28 ms, free water molecules would undergo a one-dimensional root-mean-square displacement (λ_1) of $\sim 11 \mu\text{m}$, and since the typical transverse separations between myelin sheaths in normal nerve are less than λ_1 in general, irrespective of axonal or extraaxonal environments, diffusing water molecules are expected to have numerous encounters with membranes. The 4.5 fold reduction in $D_{\text{NMR}}(\perp)$ from its free water value (see Table 6.1) is therefore clearly a characterization of the structural geometry of these multiple membrane structures in normal nerve, when compared with the 20% reduction for $D_{\text{NMR}}(\parallel)$, which presumably reflects the dearth of barriers to parallel diffusion.

a)



b)

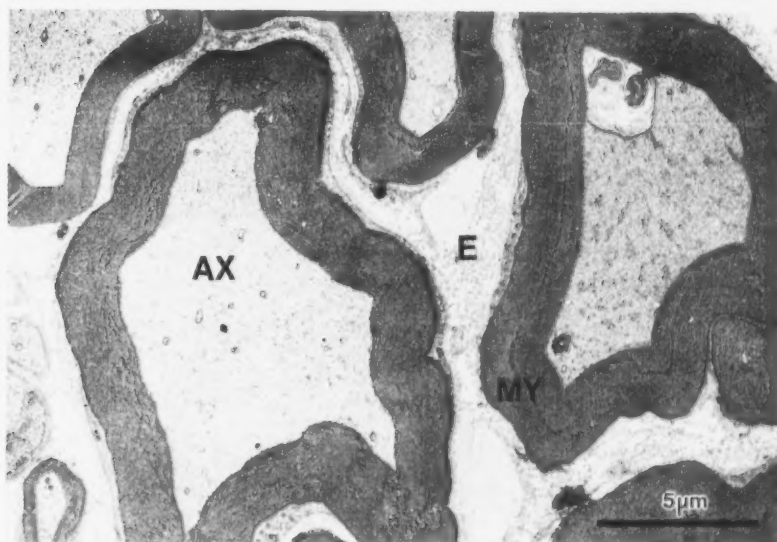


Figure 6.1 Typical light (a) and electron (b) micrographs of a transverse section of a portion of a normal sciatic nerve from the frog. (a) Numerous intact axons with their myelin sheaths are visible. The horizontal bar in the bottom, right corner represents 50 μm . (b) Several axons (AX) (~ 6 and $8 \mu\text{m}$ in diameter) in normal sciatic nerve are demonstrated. The myelin (MY) is $\sim 2 \mu\text{m}$ thick. The separation between the exterior surfaces of the adjacent myelin sheaths (which constitutes the extracellular space (E)) tends to be $\sim 0.5 - 2 \mu\text{m}$. Numerous neurofilaments fill the axonal space.

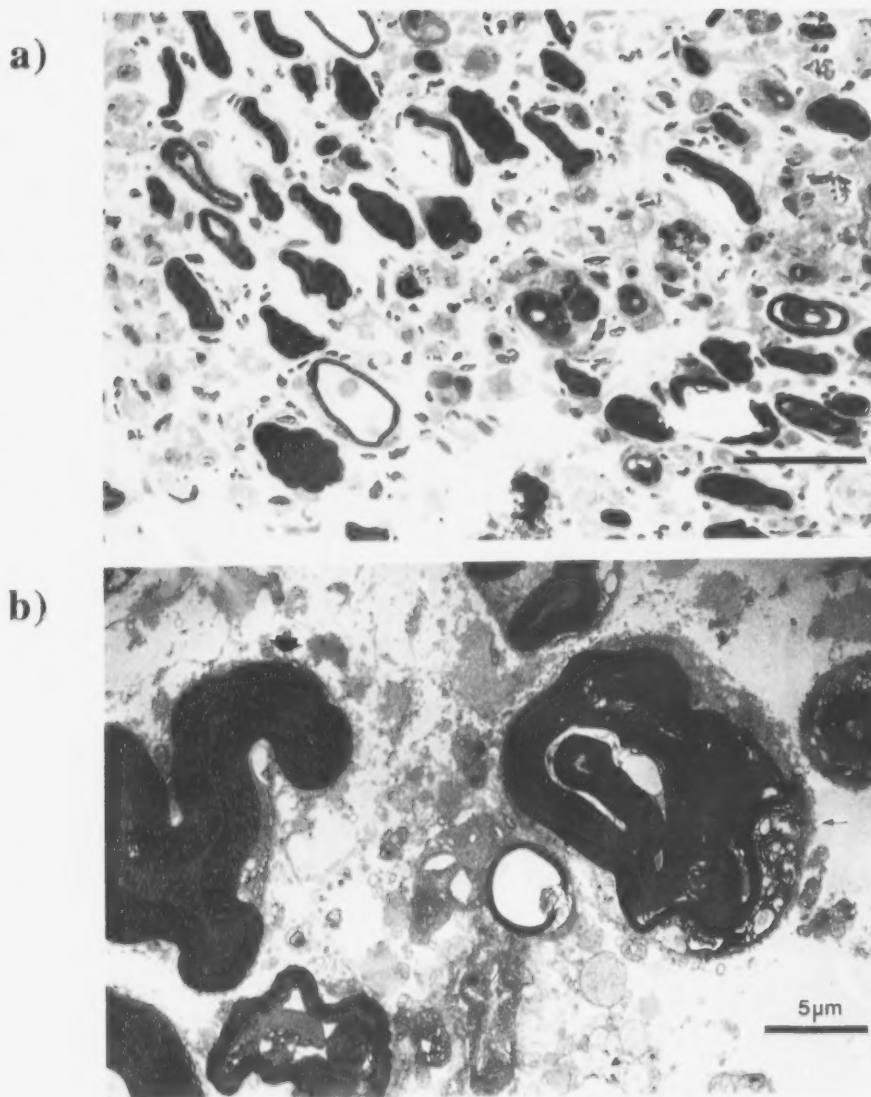
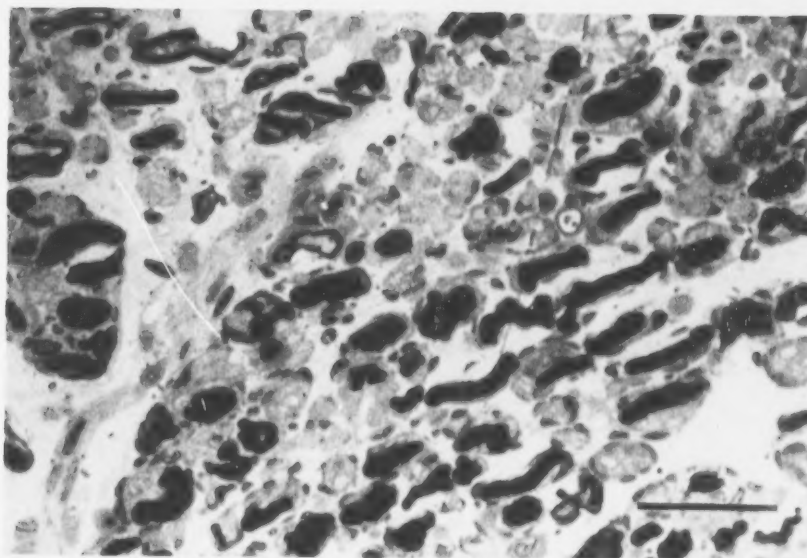


Figure 6.2 Typical light (a) and electron (b) micrographs of a transverse section of a portion of an injured sciatic nerve from the frog at 28 days post-tie. (a) There is a significant decrease in the number of myelinated fiber profiles. Any myelin that has endured appears to have collapsed. The horizontal bar in the bottom, right corner represents 50 μm . (b) There is an obvious loss of structure which usually forms longitudinal channels in the intra- and extra-cellular space of normal nerve (Fig. 6.1b). In one case, myelin has collapsed but it has retained its lamellar structure (thick arrow). In another case, the myelin is being degraded into small ovoids (thin arrow). Due to phagocytosis of axonal and myelin debris, large spaces between remaining structural masses are evident. Regenerating axon bundles are not observed in the tie-injured nerves.

a)



b)



Figure 6.3 Typical light (a) and electron (b) micrographs of a transverse section of a portion of an injured sciatic nerve from the frog at 28 days post-crush. (a) The appearance of a crush-injured nerve is similar to the tie-injured nerve seen in Fig 6.2a. The horizontal bar in the bottom, right corner represents 50 μm . (b) A collapsed myelin globule (G) is visible. An axon (3 μm diameter) has been degraded (i.e. no neurofilaments within the axoplasm) and its ensheathing myelin is being fractured and degraded by an adjacent Schwann cell (thin arrow). Groups of regenerating axons (thick arrow) are present in the crush-injured nerves.

In the tie- and crush-injured nerves, most of the axons have either collapsed or otherwise degraded (Figs. 6.2, 6.3), and are permeated with amorphous, granular material, instead of the neurofilaments and mitochondria which were clearly visible in the normal axons shown in Fig. 6.1b. The collapse of the myelin, a good deal of which is fractured and broken down by macrophages and Schwann cells (Fig. 6.3b), gives rise to an increase in the inter-myelin spaces (compare Fig. 6.1 with Figs. 6.2 and 6.3) and therefore a decrease in the obstructions for transverse diffusion, that is dependent on the length of time which phagocytosis has had to produce "open" spaces between clusters of cellular remnants (i.e. dependent on the degree of degeneration). The obstructions to parallel diffusion are correspondingly increased by the breakdown of the longitudinal axonal structure (see Fig. 2 in Schlaepfer 1964). After 28 days post-injury, the transverse separations between structural remnants typically range from 3 to 9 μm which is much larger than the 0.5 - 2 μm separations observed in normal nerve. Relative to the diffusion coefficients in normal nerve, the tie-injury degenerated state is characterized by a 27% decrease in $D_{\text{NMR}}(\parallel)$ and by a 64% increase in $D_{\text{NMR}}(\perp)$. The micrographs of the crush-injured nerves (Fig. 6.3) show degeneration that is similar to that following tie-injury. However, in the crush-injured nerve, a number of small, nonmyelinated regenerating axons could be detected (Fig. 6.3b) in the longitudinal columns formed by the bands of Büngner which provide pathways for the regenerating axons (Schroder 1975). At 28 days post-injury the changes in diffusion coefficients in the crush-injured nerves are less pronounced than those produced by the tie-injury, possibly mitigated by the regenerating axons, and give rise to significantly more anisotropy (2.3 ± 0.4) than was measured for tie-injured nerve (1.7 ± 0.1). However, in explaining the difference in outcomes between tie- and crush-injuries one must not lose sight of the possibility that different rates of the removal of longitudinally oriented barriers between the two injury models may also contribute to the observed difference in anisotropy.

For in-vivo measurements, where the relative orientation of the laboratory frame of reference and local tissue-structure frames are not easily defined, average D_{NMR} measures have been proposed. For example, to arrive at a measure of diffusion which is independent of relative frame rotations, it has been proposed (van Gelderen 1994a) that the trace of the diffusion tensor ($\mathbf{D}$) be used to define an average diffusion coefficient, D_{av} , given by

$$D_{av} = 1/3 \text{Tr}(\mathbf{D}) = 1/3 \{D(//) + 2 D(\perp)\},$$

where $\text{Tr}(\mathbf{D})$ represents the trace of the diffusion tensor. However, the opposite direction of the degeneration induced changes in the two orthogonal tissue-structure-frame diffusion coefficients gives rise to a substantially reduced sensitivity of such average measures to that degeneration. Using the diffusion coefficients from Table 6.1, D_{av} values of $(0.83 \pm 0.06) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $(0.88 \pm 0.02) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, and $(0.82 \pm 0.12) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ are calculated for the normal, tie-injured, and crush-injured nerves, respectively. In this model of degeneration D_{av} is clearly not able to distinguish between normal and degenerated nerve because the decreases in $D_{\text{NMR}}(//)$ are cancelled by the increases in $D_{\text{NMR}}(\perp)$. In fact, to differentiate between normal and degenerated nerve tissue, the data presented here confirm the importance of measuring diffusion coefficient anisotropy in the local frame of the nerve fiber tracts, or conversely, to measure the full diffusion tensor and calculate eigenvalues that correspond to the diffusion coefficients in orthogonal directions with respect to the tissue frame (Basser 1994b).

CHAPTER 7

Magnetization Transfer in Normal Myelinated and Nonmyelinated Nerves of the Garfish ⁸

7.1 Introduction

Magnetization transfer (MT) between water molecules and the macromolecular matrix of tissue (Wolff 1989) has promised to provide an alternative and in some cases possibly a more informative contrast mechanism in magnetic resonance imaging (MRI) than either longitudinal (T_1) or transverse (T_2) relaxation weighting. For example, there has been significant interest in MT contrast as a means to characterize white matter abnormalities such as those associated with multiple sclerosis (Dousset 1992, Tomiak 1994, Gass 1994). In particular, it has been reported that MT could improve MRI specificity by distinguishing edema from demyelination in lesions of white matter (Dousset 1992). The degree of MT in multiple sclerosis plaques (demyelinated) was found to be lower than the degree of MT in white matter lesions (edematous, still myelinated) of guinea pigs induced with experimental allergic encephalomyelitis, presumably due to the loss of myelin (and its constituent macromolecules) within the multiple sclerosis plaques.

To help clarify the interpretation of MT in white matter, and in particular to explore the role played by myelin as a structural element in facilitating magnetization transfer, several MT characteristics of excised, normal myelinated and nonmyelinated nerves have been measured. The nonmyelinated olfactory nerve and the myelinated trigeminal and optic nerves of the garfish were used as our simplified white matter model. In addition to typical MT values obtained at steady-state, the approach to that steady-state was considered by calculating the apparent rate constant, k_{app} , (by measuring T_1 in the presence of saturation, i.e. T_{1fsat}) and by measuring the dependence of the magnetization as a function of the length of the irradiation radiofrequency (rf) pulse. Measurements of magnetization transfer in 2 and 4% agar gels were performed prior to the measurements on nerve to ensure the veracity of our

⁸ A version of this chapter has been presented at a conference. C. Beaulieu, P.S. Allen, Some magnetization transfer properties of water in myelinated and nonmyelinated nerves. *In* Book of Abstracts, 2nd Annual Meeting of the Society of Magnetic Resonance, San Francisco, p. 169 (1994). Presented as a talk.

MT measurement methodology. The theory of magnetization transfer and a fuller description of the aforementioned parameters were discussed in Section 2.1.6 and the experimental methodology was discussed in Section 3.3.3.

7.2 Results and Discussion

To standardize the MT methodology, magnetization transfer amplitudes were measured in 2 and 4% agar gels. Knowing the T_1 and T_2 of the agar gel samples (see Table 7.1), deviations from the offset frequency ($\Delta\omega$) dependence that is expected for direct saturation (given by Eqn. 2.69) allow an estimation of MT amplitude to be made for each gel (see Fig. 7.1). The similarity of the maximum magnetization transfer amplitudes reported in Table 7.2 demonstrates the lack of a linear dependence between magnetization transfer amplitudes and macromolecule concentration in the agar gels. However, the relative values of k_{app} (see Table 7.2) are in line with the concentration ratio, though the *absolute* values are somewhat lower than those calculated by curve-fitting procedures, i.e. $0.9 \pm 0.1 \text{ s}^{-1}$ and $1.8 \pm 0.2 \text{ s}^{-1}$ for 2 and 4% agar gels (Henkelman 1993, RM_0^b in their terminology), presumably due to incomplete saturation of the protons of the macromolecular matrix (Henkelman 1993, Brooks 1994). Similarly to others (Brooks 1994, Kucharczyk 1994), we have found that the measured k_{app} values in the agar gels (and the garfish nerves) are dependent upon the rf strength (B_2) and offset frequency of the irradiation pulse (unpublished results). Nevertheless, using the $\Delta\omega$, B_2 combination of irradiation parameters that yield the maximum degree of MT, the *relative* values of k_{app} appear to yield useful and relevant information. Furthermore, the measurement of *relative* rates is likely adequate since the calculation of *true* rates would require the use of more sophisticated curve-fitting routines on more abundant data sets (Henkelman 1993).

Table 7.1 Relaxation characteristics of water in 2 and 4% agar gels and in the olfactory, trigeminal, and optic nerves of the garfish.

Sample	T_1 (s) ^b	T_2 (s)	$\frac{T_1}{T_2}$
Phantoms (N=2) ^a			
2% agar gel	2.28 ± 0.02	0.072 ± 0.005	32 ± 2
4% agar gel	2.03 ± 0.06	0.039 ± 0.003	53 ± 5
Garfish nerves (N=4)			
Olfactory	1.56 ± 0.04	0.147 ± 0.004 ^c	10.6 ± 0.1
Trigeminal	1.17 ± 0.03	0.137 ± 0.005 ^c	8.6 ± 0.2
Optic	1.01 ± 0.09	0.115 ± 0.006 ^c	8.8 ± 1.0

^a The average $\pm$ standard deviation for measurements on N samples.

^b T_1 is measured using a routine inversion recovery pulse sequence.

^c The T_2 values are one component fits of the CPMG data even though the CPMG relaxation decay curves are multiexponential.

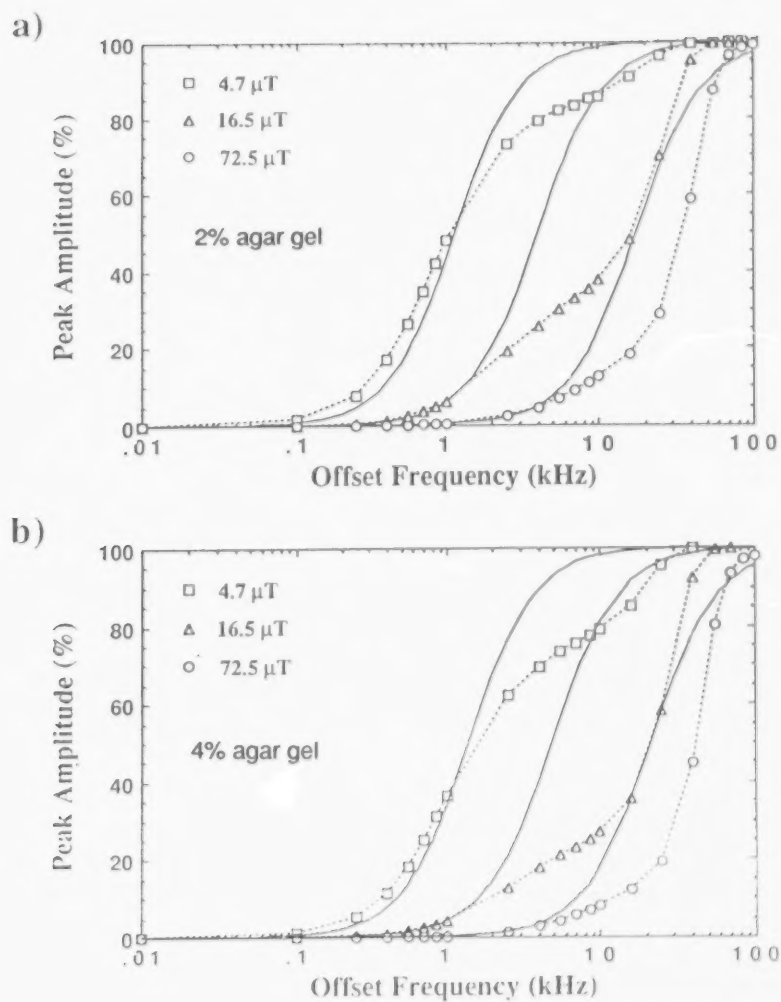


Figure 7.1 Figure caption on next page.

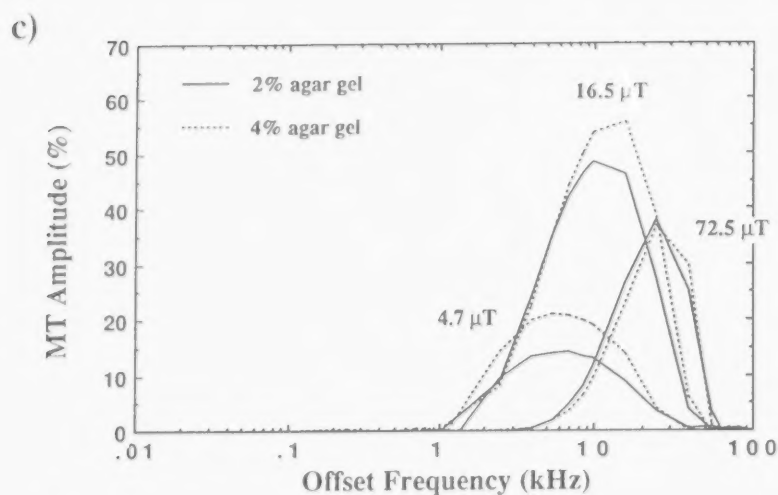


Figure 7.1 Peak amplitudes of water in 2% (a) and 4% (b) agar gels for three rf strengths of the irradiation rf pulse at various offset frequencies. Marked deviations from the direct saturation curves (solid lines calculated from Eqn. 2.69) are evident. The difference between the calculated direct saturation curves and the experimental curves yields a measure of the signal loss due to MT solely (i.e. MT amplitude) (c). For each rf strength, there is an offset frequency that yields the maximum MT amplitude. An offset frequency of ~ 10 kHz for an rf strength of $\sim 16.5 \mu\text{T}$ yields the largest MT amplitude for both gels.

Table 7.2 Some magnetization transfer properties of water in agar gels and garfish nerves measured using an irradiation rf strength (B_2) of 16.5 μ T at an offset frequency ($\Delta\omega$) of 10 kHz ^a.

Sample	MT Amp (%) ^c	T_{1f} (s) ^d	T_{1fsat} (s)	k_{app} (s ⁻¹) ^e
Phantoms (N=2) ^b				
2% agar gel	46.4 $\pm$ 2.8	2.42 $\pm$ 0.25	0.91 $\pm$ 0.09	0.71 $\pm$ 0.08
4% agar gel	54.8 $\pm$ 1.3	2.10 $\pm$ 0.10	0.53 $\pm$ 0.02	1.41 $\pm$ 0.07
Garfish nerves (N=4)				
Olfactory	54.3 $\pm$ 1.2	1.77 $\pm$ 0.09	0.75 $\pm$ 0.02	0.80 $\pm$ 0.04
Trigeminal	53.0 $\pm$ 3.2	1.54 $\pm$ 0.05	0.70 $\pm$ 0.06	0.83 $\pm$ 0.09
Optic	59.0 $\pm$ 0.4	1.31 $\pm$ 0.14	0.50 $\pm$ 0.05	1.30 $\pm$ 0.14

^a This combination of B_2 and $\Delta\omega$ was chosen since it yields the largest MT amplitude (approximately) for the gels and nerves (see Fig. 7.2).

^b The average $\pm$ standard deviation are shown for the measurements on N samples.

^c The MT amplitude is derived from the difference between the measured MT curves and the calculated direct saturation curves.

^d T_{1f} is calculated using Eqn. 2.68.

^e k_{app} is calculated using Eqn. 2.66.

A detailed illustration of the data from the garfish nerves is provided in Figs. 7.2a - c where the offset dependence of the water signal amplitude is shown in comparison with the effect of direct saturation as predicted by Eqn. 2.69 at three different rf strengths, namely, 4.7, 16.5, and 72.5 μ T. As a consequence of MT, significant additional signal loss beyond direct saturation is observed. This additional MT signal loss is shown for all three garfish nerves at the three rf strengths in Fig. 7.2d and its maximum amplitudes, given in Table 7.2, are to be compared with a direct saturation loss of only $\sim$ 4 - 5% for the irradiation parameters which yield the maximum MT, i.e. $\Delta\omega$ = 10 kHz and B_2 = 16.5 μ T. The optimization of irradiation rf parameters which maximize MT is important for delineating MR imaging sequence parameters that will optimize the sensitivity of the image to changes in MT caused by tissue pathology. An MT amplitude of 42% was measured in normal human white matter in-vivo using $\Delta\omega$ = 2 kHz and B_2 = 3.7 μ T (Dousset 1992). The closest

matching irradiation parameters in the olfactory, trigeminal, and optic nerves of the garfish, namely, $\Delta\omega = 2.5$ kHz and $B_2 = 4.7$ μ T gave rise to MT amplitudes of 42, 43, and 48%, which are in good agreement with the human white matter data. This agreement, while lending credibility to the excised nerve model, also suggests that a better choice of irradiation parameters might have been made for the white matter. The optimal values for garfish nerve are encouragingly similar to those found for some model lipid systems and for bovine white matter at a field strength of 1.5 T (Kucharczyk 1994).

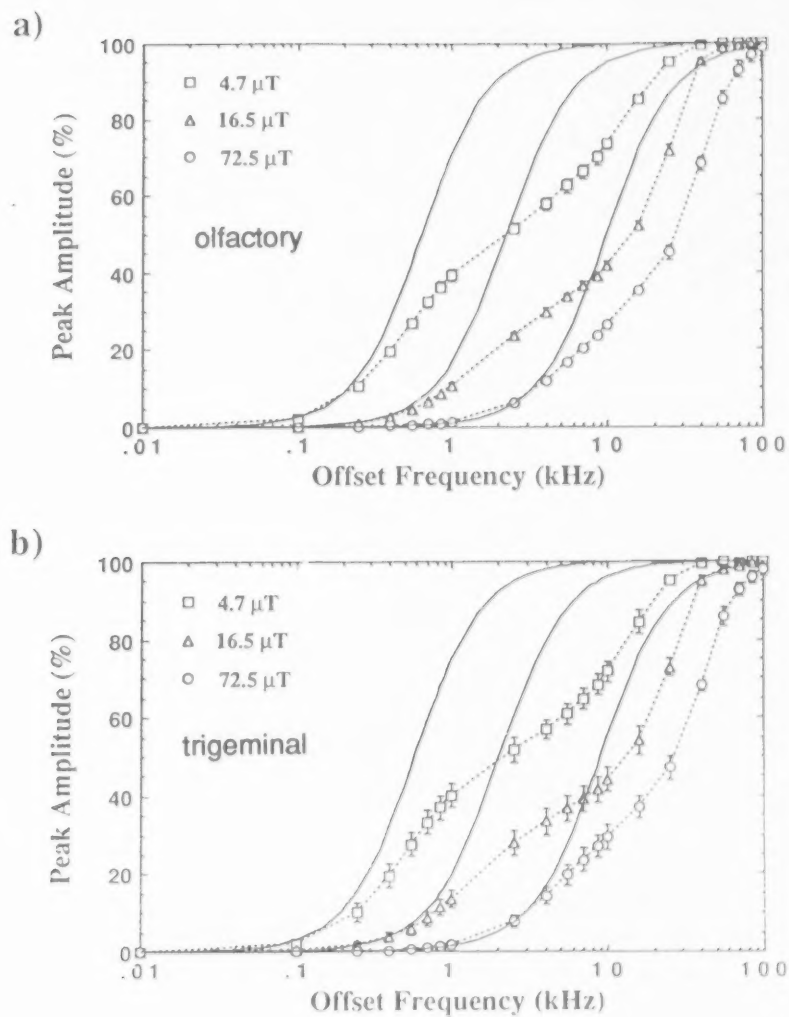


Figure 7.2 Figure caption on next page.

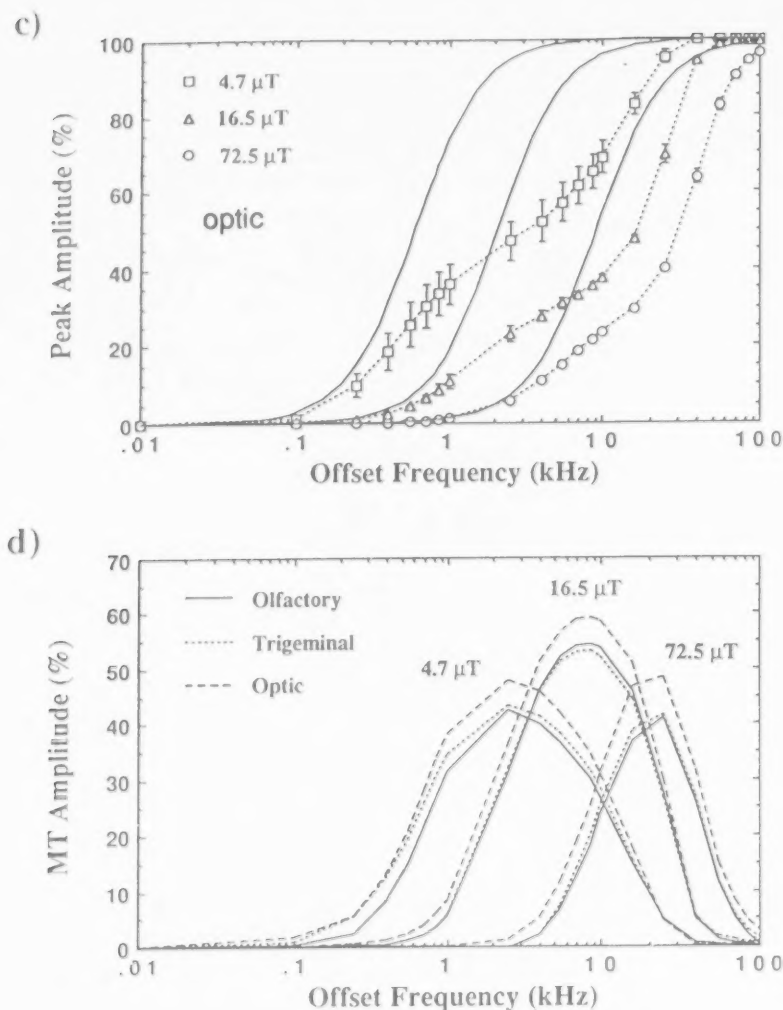


Figure 7.2 Peak amplitudes of water in the nonmyelinated olfactory (a), myelinated trigeminal (b), and myelinated optic (c) nerves averaged over four garfish for three rf strengths at various offset frequencies, the error bars reflecting the standard deviation (in some instances, the error bars do not extend past the plot symbols). The standard deviations are usually less than $\pm 3\%$. Marked deviations from the direct saturation curves (solid lines calculated from Eqn. 2.69) are evident, indicating a large degree of magnetization transfer in the nerves. The difference between the calculated direct saturation curves and the experimental curves yields a measure of the signal loss solely due to MT (i.e. MT amplitude) (d). For each rf strength, there is an offset frequency that yields the maximum MT amplitude. An offset frequency of ~ 10 kHz for an rf strength of ~ 16.5 μT yields the largest MT amplitude for all three nerves.

Because the steady-state MT amplitude does not vary greatly between the myelinated and nonmyelinated normal garfish nerves (Fig. 7.2, Table 7.2), it does not appear to be unduly sensitive to the presence of multiply wrapped membrane in the form of myelin. From the garfish data it certainly cannot be said to be specific to myelin. In fact, the membrane densities, including myelin and axonal membranes, are estimated from transverse electron micrographs of the nerves to be approximately 13, 28, and 26% for the olfactory, trigeminal, and optic nerves, respectively. Therefore, the observation of similar MT amplitudes in nerve samples with differing membrane densities, together with the uncertain contribution of other macromolecular constituents of nerve, such as axoplasmic proteins, undermines any hypothesis of a very close correspondence between steady-state MT and membrane density in nerve, even though changes in membrane density are undoubtedly reflected by changes in MT (Dousset 1992, Hiehle 1994). The lack of membrane specificity for MT in the garfish nerves is in agreement with the observed similarity of steady-state MT for such diverse systems as liver, muscle, optic nerve, white matter, and grey matter (Morrison 1995a).

In addition to the steady-state MT amplitude, the approach to that equilibrium was investigated and characterized by means of experimental T_{1fsat} values and k_{app} values calculated from Eqn. 2.66, each of which is listed in Table 7.2 for the agar gels and the garfish nerves. All these values were acquired under irradiation conditions of $\Delta\omega = 10$ kHz and $B_2 = 16.5$ μ T. Figure 7.3 illustrates the dependence of the water signal amplitude on the duration of the off-resonance irradiation. It is clear from Fig. 7.3 that 5 s irradiation was more than sufficient to achieve steady-state.

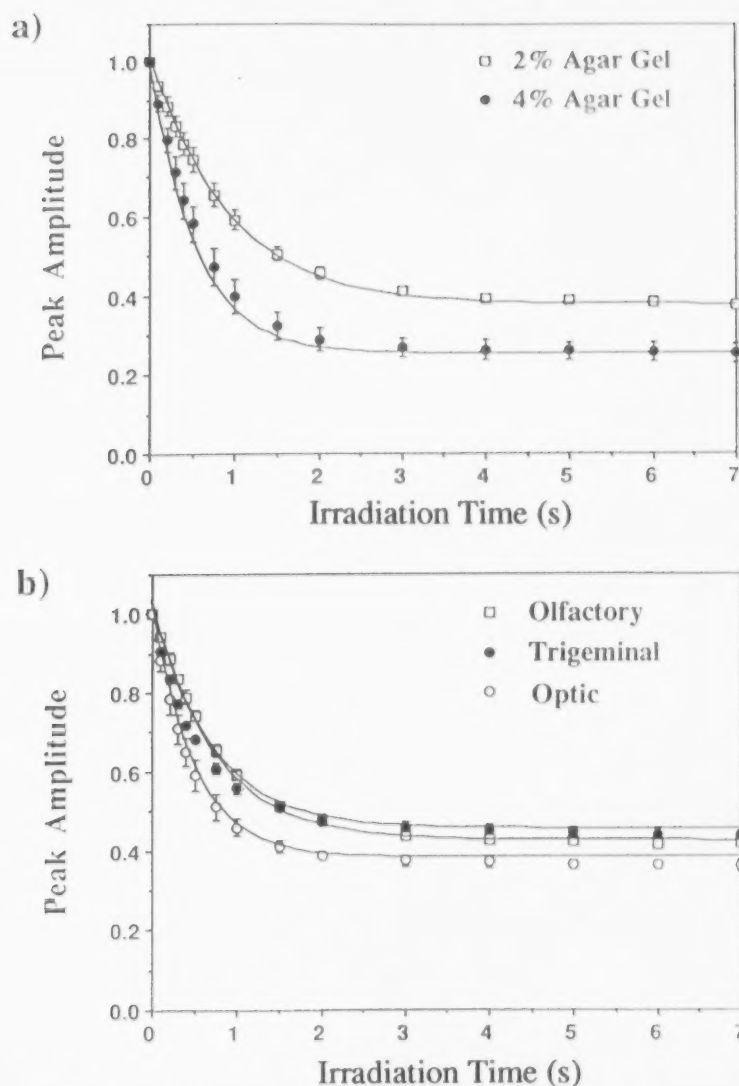


Figure 7.3 The average peak amplitudes of water in 2 and 4% agar gels (a) (two samples each) and in garfish nerves (b) (four samples each) as a function of the duration of the irradiation rf pulse ($\Delta\omega = 10$ kHz, $B_2 = 16.5 \mu\text{T}$). The error bars correspond to the standard deviations. Except for the initial part of the curve for the trigeminal nerve, there is good agreement between the experimental data points and the solid curves which are calculated from Eqn. 2.67 using the independently derived parameters found in Table 7.2 (T_{1f} , T_{1fsat} , k_{app}). The 4% agar gel reaches steady-state faster than the 2% gel. Likewise, the myelinated nerves (particularly the optic) appear to reach steady-state faster than the nonmyelinated nerve (olfactory).

The agar gel data demonstrate a change in the transfer efficiency of the magnetization, namely, an approximate doubling of k_{app} and an approximate halving of T_{1fsat} (Table 7.2) that is more in line with the concentration increase than is the change in steady-state amplitude. The approach to equilibrium may therefore be the more sensitive measure of sample composition. For the garfish nerves, the myelinated optic nerve reaches its steady-state significantly more rapidly than the nonmyelinated olfactory nerve (Table 7.2, Fig. 7.3), displaying k_{app} and T_{1fsat} values that are a factor of $\sim 1.5 - 1.7$ different than their olfactory counterparts. The corresponding steady-state MT amplitudes differ by the much smaller factor of 1.1. However, the trigeminal nerve's k_{app} and T_{1fsat} values are not different from the olfactory nerve's values to any great extent, even though the membrane density of the myelinated trigeminal nerve is about twice that of the nonmyelinated olfactory nerve. Also, the lipid compositions of the trigeminal and olfactory nerves are substantially different, the trigeminal possessing larger concentrations of cholesterol, sulfatides, and cerebroside (Holton 1971). Furthermore, the optic nerve approaches steady-state more rapidly than the trigeminal nerve despite both having similar densities of myelin. Since MT appears to be quite nonspecific (Morrison 1995a), the differences in MT may simply reflect the fractional sizes of MT active macromolecular pools (not necessarily myelin). It is not known whether or not a more efficient transfer of MT occurs when the constituents of myelin are present. Regardless of the exact mechanism for the enhanced rates, the approach to steady-state appears to be more sensitive to differences in tissue composition (Eng 1991) and may be used to improve the differentiation of tissues with similar MT levels at steady-state, even though the saturation of the macromolecular pool may be incomplete.

The three T_2 components, short, intermediate, and long (as previously discussed in Section 2.2.1), in nerve have been identified respectively with myelinic, axonal, and inter-axonal water. By applying an irradiation rf pulse prior to a CPMG pulse sequence, the degree of signal loss due to magnetization transfer was evaluated for the three T_2 components observed in the sciatic nerve of the frog⁹. Using $\Delta\omega =$

⁹ These results have been presented at a conference. M.D. Does, C. Beaulieu, P.S. Allen, R.E. Snyder, Magnetization transfer of nerve water T_2 components. *In* Book of Abstracts, 3rd Annual Meeting of the Society of Magnetic Resonance, Nice, p. 109 (1995). Presented as a talk (MD).

10 kHz and $B_2 = 16.5 \mu\text{T}$ for the saturation parameters, the degree of signal loss was 72 ± 4 , 71 ± 4 , and 44 ± 5 % for the short, intermediate, and long T_2 components. The bulk signal loss of the frog sciatic nerve was 62 ± 2 % (very similar to the garfish optic nerve). Therefore, the two shorter lived T_2 components showed greater magnetization transfer than the longer lived T_2 component. Moreover, there was no significant difference in *steady-state* MT between the two shorter lived components. This suggested that axonal and myelinic water possess similar degrees of steady-state MT, in agreement with our findings in the nonmyelinated and myelinated garfish nerves. However, recent measurements using various durations of saturation (as in Fig. 7.3) have shown that the short T_2 component approaches steady-state more rapidly than the intermediate T_2 component (Does 1995c). These results suggest that myelinic water approaches steady-state more rapidly either because the myelin macromolecules have a more efficient transfer of magnetization with the bulk water or because there is simply a greater proportion of macromolecules in the myelin than in the axoplasm. The MT differences between the individual T_2 components supports the hypothesis that these T_2 components correspond to distinct compartments of water.

CHAPTER 8

Concluding Remarks

Since magnetic resonance imaging (MRI) typically involves signal from the hydrogen nuclei of water (i.e. the protons), the ability to discriminate one tissue type from another depends on the different interactions of water within the unique macromolecular matrix that comprises each tissue. By understanding how these water properties are influenced by various cellular components in normal nervous tissue, we can better predict how the properties of water may be altered by pathology. The purpose of this thesis was to provide an understanding (where before there was little or none) of the relationship between certain NMR properties of water in tissue and the physiological microstructure of that tissue, particularly in nerve and in white matter. For the purposes of this thesis, diffusion and magnetization transfer were the two properties of water protons that were investigated in a series of white matter models.

A major portion of this thesis was dedicated to an evaluation of what diffusion measurements tell us when they expose a change in diffusion coefficient anisotropy resulting from pathology. To this end we determined, experimentally, the dominant source of anisotropic diffusion of water in nerve, and by implication, in white matter. By means of both the nonmyelinated and myelinated nerves of the garfish, it was shown that the presence of myelin was not essential for anisotropy to be observed. This is not to say, however, that myelin when it is present does not contribute to any observed anisotropy, but that it is not a prerequisite in some axonal systems. The role of other structural components in hindering water diffusion perpendicular to the length of the nerve were therefore explored. For example, by depolymerizing microtubules and inhibiting fast axonal transport in garfish nerves with vinblastine, we showed that microtubules and fast axonal transport are not significant determinants of diffusional anisotropy in garfish nerve. To evaluate the role of neurofilaments we took advantage of the minimal influence of axonal membranes in the giant axon of the squid and showed that neurofilaments do not obstruct water diffusion perpendicular to the nerve axis to any great extent either. Membranes therefore remain the only viable candidate for diffusional anisotropy. However, there are two possible ways in which membranes may give rise to an anisotropic diffusion coefficient. The first is by inducing local susceptibility-difference (i.e. background) gradients through which the

water will diffuse. Because we are able to align the nerves parallel or perpendicular to the static magnetic field, we were able to show that the background gradients do not contribute to the anisotropy of measured diffusion coefficients at magnetic field strengths ≤ 2.35 T. The second and only remaining way that membranes, either in the form of multiply wrapped myelin or of single axonal membranes, can provide the primary mechanism for anisotropic diffusion of water is through their inhibition of transverse diffusion at each membrane encounter. Such a mechanism explains the anisotropy observed in both myelinated and nonmyelinated nerve and is reinforced by the two-fold increase in the transverse diffusion coefficient as the diffusion time was decreased from 28 ms to 2 ms (i.e. water had fewer encounters with the inhibiting membrane structures at the shorter diffusion time). The above series of experiments confirmed the hypothesis that in myelinated nerves, myelin was likely the dominant source of anisotropic water diffusion.

Previous work from this laboratory had shown that water in garfish nerve could be compartmentalized on the basis of distinguishable T_2 components and that the amplitude of these components bore a close relation to the water content of certain physical structures of the nerve. In an attempt to identify these compartments by means of another NMR parameter, the diffusion characteristics of the long-lived T_2 component were evaluated. Assuming the previous assignment, namely, that the long T_2 component corresponds to water in the inter-axonal space, the water diffusion in the inter-axonal space was found to be hindered and anisotropic. This result is not surprising if one considers that even the water in the inter-axonal spaces is confined in the transverse direction between the membranes of a vast number of packed axons. It was not possible to differentiate the diffusion characteristics of other water compartments.

The sensitivity of diffusion measurements to tissue pathology is of the utmost importance. The detection and characterization of the loss and/or breakdown of axonal membranes and myelin was therefore addressed in a study of the degeneration of injured peripheral nerve (i.e. sciatic nerve of the frog). It was found that the root-mean-square displacement of water, and hence the measured diffusion coefficients, along the length of the degenerated nerves was reduced from that in normal nerves due to the blockage of the longitudinal channels by degenerating debris, whereas the diffusion coefficients perpendicular to the nerve axis increased due to the loss of

membrane integrity. These results are suggestive of the possibility that a quantitative anisotropy measure may be devised that allows one to monitor the state of degeneration in nerve in-vivo. An interesting aspect of this work was our observation that the anisotropy observed in degenerating nerves seemed to reflect the presence of regenerating axons. Future studies should evaluate the efficacy of anisotropic diffusion for detecting nerve regeneration in appropriate animal models.

In addition to its effect on water diffusion, the myelin in white matter has been associated previously with the observation of a significant amount of magnetization transfer from the water protons. In view of the understanding gained from our earlier experiments concerning the significance of myelin in the observation of diffusional anisotropy, a similar evaluation of its significance for magnetization transfer was deemed appropriate. The myelinated and nonmyelinated nerves of the garfish were again used as a model system, giving rise to similar magnetization transfer effects. Components of nerve, other than myelin, are sufficient to generate significant magnetization transfer. Therefore, in a similar conclusion to the measurements of anisotropic diffusion presented above, efficient magnetization transfer in nerve is not *specific* to myelin, despite the blanket statements to the contrary that are present in the literature (Grossman 1994). An additional outcome of the present work has been the appreciation of the dynamics of the transfer process as being more sensitive to structural differences than the steady state conditions.

Given the growing utility of diffusion-weighted MRI for the early detection of acute stroke and of magnetization transfer for the improved detection of demyelinating lesions, the improved understanding of diffusion and magnetization transfer in nerve resulting from the work presented in this thesis will hopefully provide a clearer picture of these water properties for use in the management of white matter disease.

REFERENCES

- Abragam A., "Principles of Nuclear Magnetism", Oxford University Press, Oxford, 1961a.
- Abragam A., "Principles of Nuclear Magnetism", pp. 45-46, Oxford University Press, Oxford, 1961b.
- Adelman W.J., D.L. Gilbert, Electrophysiology and biophysics of the squid giant axon, *In "Squid as Experimental Animals,"* (W.J. Adelman, D.L. Gilbert, J.M. Arnold, Eds.), pp. 103-112, Plenum Press, New York and London, 1990.
- Alger J.R., R.G. Shulman, NMR studies of enzymatic rates in vitro and in vivo by magnetization transfer. *Quart Rev Biophys* **17**, 83 (1984).
- Anderson A.W., J.C. Gore, Analysis and correction of motion artifacts in diffusion weighted imaging. *Magn Reson Med* **32**, 379 (1994).
- Atkins P.W., "Physical Chemistry", 3rd Ed., pp. 336-337 WH Freeman and Company, New York, 1986a.
- Atkins P.W., "Physical Chemistry", 3rd Ed., pp. 681-683, WH Freeman and Company, New York, 1986b.
- Baker P.F., A.C. Crawford, Mobility and transport of magnesium in squid giant axons. *J Physiol* **227**, 855 (1972).
- Balaban R.S., T.L. Ceckler, Magnetization transfer contrast in magnetic resonance imaging. *Magn Reson Quart* **8**, 116 (1992).
- Barrow G.M., "Physical Chemistry", 4th Ed., pp. 765-771, McGraw Hill Book Company, New York, 1979.
- Basser P.J., J. Mattiello, D. Le Bihan, Estimation of the self-diffusion tensor from the NMR spin echo. *J Magn Reson B* **103**, 247 (1994a).
- Basser P.J., J. Mattiello, D. Le Bihan, MR diffusion tensor spectroscopy and imaging. *Biophys J* **66**, 259 (1994b).
- Beneviste H., L.W. Hedlund, G.A. Johnson, Mechanism of detection of acute cerebral ischemia in rats by diffusion-weighted magnetic resonance microscopy. *Stroke* **23**, 746 (1992).
- Bensch K.G., S.E. Malawista, Microtubular crystals in mammalian cells. *J Cell Biol* **40**, 95 (1969).
- Berndt K.D., J. Beunink, W. Schroder, K. Wuthrich, Designed replacement of an internal hydration water molecule in BPTI: Structural and functional implications of a glycine-to-serine mutation. *Biochemistry* **32**, 4564 (1993).

- Bizzi A., A. Righini, R. Turner, D. LeBihan, D. DesPres, G. Di Chiro, J.R. Alger, MR of diffusion slowing in global cerebral ischemia. *AJNR* **14**, 1347 (1993).
- Bloch F., Nuclear induction. *Phys Rev* **70**, 460 (1946a).
- Bloch F., W.W. Hansen, M. Packard, Nuclear induction. *Phys Rev* **69**, 127 (1946b).
- Bloembergen N., E.M. Purcell, R.V. Pound, Relaxation effects in nuclear magnetic resonance absorption. *Phys Rev* **73**, 679 (1948).
- Boorstein J.M., K.T. Wong, R.I. Grossman, L. Bolinger, J.C. McGowan, Metastatic lesions of the brain: imaging with magnetization transfer. *Radiology* **191**, 799 (1994).
- Bovey F.A., "Nuclear Magnetic Resonance Spectroscopy", pp. 255-257, Academic Press, San Diego, 1988.
- Brooks D., K. Kuwata, T. Schleich, Determination of proton magnetization transfer rate constants in heterogeneous biological systems. *Magn Reson Med* **31**, 331 (1994).
- Brown A., R.J. Lasek, The cytoskeleton of the squid giant axon, *In* "Squid as Experimental Animals," (W.J. Adelman, D.L. Gilbert, J.M. Arnold, Eds.), pp. 235-302, Plenum Press, New York and London, 1990.
- Bunch W.H., G. Kallsen, Rate of intracellular diffusion as measured in barnacle muscle. *Science* **164**, 1178 (1969).
- Busza A.L., K.L. Allen, M.D. King, N. van Bruggen, S.R. Williams, D.G. Gadian, Diffusion-weighted imaging studies of cerebral ischemia in gerbils: potential relevance to energy failure. *Stroke* **23**, 1602 (1992).
- Caines G.H., T. Schleich, Incorporation of saturation transfer in the formalism of rotating-frame spin-lattice NMR relaxation in the presence of an off-resonance-irradiation field. *J Magn Reson* **95**, 457 (1991a).
- Caines G.H., T. Schleich, J.M. Rydzewski, Incorporation of magnetization transfer into the formalism for rotating-frame spin-lattice NMR relaxation in the presence of an off-resonance-irradiation field. *J Magn Reson* **95**, 558 (1991b).
- Callaghan P.T., Pulsed field gradient nuclear magnetic resonance as a probe of liquid state molecular organization. *Aust J Phys* **37**, 359 (1984).
- Callaghan P.T., C.D. Eccles, Y. Xia, NMR microscopy of dynamic displacements: k-space and q-space imaging. *J Phys E: Sci Instrum* **21**, 820 (1988).
- Callaghan P.T., "Principles of Nuclear Magnetic Resonance Microscopy", pp. 157-172, 328-370, Clarendon Press, Oxford, 1991a.

- Callaghan P.T., Y. Xia, Velocity and diffusion imaging in dynamic NMR microscopy. *J Magn Reson* **91**, 326 (1991b).
- Cancalon P., The relationship of slow axonal flow to nerve elongation and demyelination, In "Axonal Transport in Neuronal Growth and Regeneration" (J.S. Elam, P. Cancalon, Eds.), pp. 211-241, Plenum Press, New York, 1984.
- Carr H.Y., E.M. Purcell, Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Phys Rev* **94**, 630 (1954).
- Ceckler T.L., S.D. Wolff, V. Yip, S.A. Simon, R.S. Balaban, Dynamic and chemical factors affecting water proton relaxation by macromolecules. *J Magn Reson* **98**, 637 (1992).
- Chan H., R.S. Smith, R.E. Snyder, Junction between parent and daughter axons in regenerating myelinated nerve: properties of structure and rapid axonal transport. *J Comp Neurol* **283**, 391 (1989).
- Chang D.C., H.E. Rorschach, B.L. Nichols, C.F. Hazlewood, Implications of diffusion coefficient measurements for the structure of cellular water. *Ann NY Acad Sci* **204**, 434 (1973).
- Chang D.C., C.F. Hazlewood, Nuclear magnetic resonance study of squid giant axon. *Biochim Biophys Acta* **630**, 131 (1980).
- Chenevert T.L., J.A. Brunberg, J.G. Pipe, Anisotropic diffusion in human white matter: demonstration with MR techniques in vivo. *Radiology* **177**, 401 (1990).
- Chenevert T.L., B.D. Ross, J.G. Pipe, S.J. Simerville, Quantitative diffusion anisotropy in rat gliomas. In Book of Abstracts, 10th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 787 (1991).
- Chien D., R.B. Buxton, K.K. Kwong, T.J. Brady, B.R. Rosen, Quantitative diffusion imaging in the human brain. In Book of Abstracts, 7th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 218 (1988).
- Chien D., R.B. Buxton, K.K. Kwong, T.J. Brady, B.R. Rosen, MR diffusion imaging of the human brain. *J Comput Assist Tomogr* **14**, 514 (1990).
- Chien D., K.K. Kwong, D.R. Gress, F.S. Buonanno, R.B. Buxton, B.R. Rosen, MR diffusion imaging of cerebral infarction in humans. *AJNR* **13**, 1097 (1992).
- Christiansen P., P. Gideon, C. Thomsen, M. Stubgaard, O. Henriksen, H.B.W. Larsson, Increased water self-diffusion in chronic plaques and in apparently normal white matter in patients with multiple sclerosis. *Acta Neurol Scand* **87**, 195 (1993).

- Cleveland G.G., D.C. Chang, C.F. Hazlewood, H.E. Rorschach, Nuclear magnetic resonance measurement of skeletal muscle. Anisotropy of the diffusion coefficient of the intracellular water. *Biophys J* **16**, 1043 (1976).
- Cohen Y., J. Mintorovitch, L. Chileuitt, B. Pereira, H. Shimizu, P. Weinstein, M.E. Moseley, Early detection of ischemic injury: comparison of diffusion- and T₂-weighted MRI and spectroscopy during regional cerebral ischemia in cats. *In* Book of Abstracts, 8th Annual Meeting of the Society of Magnetic Resonance in Medicine, Amsterdam, p. 42 (1989).
- Cooper R.L., D.B. Chang, A.C. Young, C.J. Martin, B. Ancker-Johnson, Restricted diffusion in biophysical systems. *Biophys J* **14**, 161 (1974).
- Cope F.W., Nuclear magnetic resonance evidence using D₂O for structured water in muscle and brain. *Biophys J* **9**, 303 (1969).
- Coremans J., R. Luybaert, F. Verhelle, T. Stadnik, M. Osteaux, A method for myelin fiber orientation mapping using diffusion-weighted MR images. *Magn Reson Imaging* **12**, 443 (1994).
- Cory D.G., A.N. Garroway, Measurements of translational displacement probabilities by NMR: an indicator of compartmentation. *Magn Reson Med* **14**, 435 (1990).
- Cotts R.M., M.J.R. Hoch, T.Sun, J.T. Markert, Pulsed field gradient stimulated echo methods for improved NMR diffusion measurements in heterogeneous systems. *J Magn Reson* **83**, 252 (1989).
- Damji A.K., Personal communication, 1995.
- Dardzinski B.J., C.H. Sotak, M. Fisher, Y. Hasegawa, L. Li, K. Minematsu, Apparent diffusion coefficient mapping of experimental focal cerebral ischemia using diffusion-weighted echo-planar imaging. *Magn Reson Med* **30**, 318 (1993).
- Davis D., J. Ulatowski, S. Eleff, M. Izuta, S. Mori, D. Shungu, P.C.M. van Zijl, Rapid monitoring of changes in water diffusion coefficients during reversible ischemia in cat and rat brain. *Magn Reson Med* **31**, 454 (1994).
- de Crespigny A.J., M. Tsuura, M.E. Moseley, J. Kucharczyk, Perfusion and diffusion MR imaging of thromboembolic stroke. *J Magn Reson Imag* **3**, 746 (1993).
- de Crespigny A.J., M.P. Marks, D.R. Enzmann, M.E. Moseley, Navigated diffusion imaging of normal and ischemic human brain. *Magn Reson Med* **33**, 720 (1995).
- Derome A.E., "Modern NMR Techniques for Chemistry Research", pp. 98-101, Pergamon Press, Oxford, 1987.

- Does M.D., R.E. Snyder, Multi-component T_2 changes in degenerating peripheral nerve measured in vivo. *In Book of Abstracts, 2nd Annual Meeting of the Society of Magnetic Resonance, San Francisco, p. 199 (1994).*
- Does M.D., R.E. Snyder, Multi-exponential T_2 relaxation in degenerating peripheral nerve. *Magn Reson Med* **submitted** (1995a).
- Does M.D., R.E. Snyder, T_2 relaxation of peripheral nerve measured in vivo. *Magn Reson Imaging* **in press** (1995b).
- Does M.D., Personal communication, 1995c.
- Doran M., J.V. Hajnal, N. van Bruggen, M.D. King, I.R. Young, G.M. Bydder, Normal and abnormal white matter tracts shown by MR imaging using directional diffusion weighted sequences. *J Comput Assist Tomogr* **14**, 865 (1990).
- Douek P., R. Turner, J. Pekar, N. Patronas, D. LeBihan, MR color mapping of myelin fiber orientation. *J Comput Assist Tomogr* **15**, 923 (1991).
- Dousset V., R.I. Grossman, K.N. Ramer, M.D. Schnall, L.H. Young, F. Gonzalez-Scarano, E. Lavi, J.A. Cohen, Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. *Radiology* **182**, 483 (1992).
- Easton D.M., Impulses at the artifactual nerve end. *Cold Spring Harbor Symposium on Quantitative Biology* **30**, 15 (1965).
- Easton D.M., Garfish olfactory nerve: easily accessible source of numerous long, homogeneous, nonmyelinated axons. *Science* **172**, 952 (1971).
- Ebisu T., S. Naruse, Y. Horikawa, S. Ueda, C. Tanaka, M. Uto, M. Umeda, T. Higuchi, Discrimination between different types of white matter edema with diffusion-weighted MR imaging. *J Magn Reson Imag* **3**, 863 (1993).
- Edelman R.R., S.S. Ahn, D. Chien, W. Li, A. Goldmann, M. Mantello, J. Kramer, J. Kleefield, Improved time-of-flight MR angiography of the brain with magnetization transfer contrast. *Radiology* **184**, 395 (1992).
- Edzes H.T., E.T. Samulski, Cross relaxation and spin diffusion in the proton NMR of hydrated collagen. *Nature* **265**, 521 (1977).
- Edzes H.T., E.T. Samulski, The measurement of cross-relaxation effects in the proton NMR spin-lattice relaxation of water in biological systems: hydrated collagen and muscle. *J Magn Reson* **31**, 207 (1978).
- Eisenberg D., W. Kauzmann, "The Structure and Properties of Water", p. 179, Oxford University Press, Oxford, 1969.
- Eng J., T.L. Ceckler, R.S. Balaban, Quantitative ^1H magnetization transfer imaging in vivo. *Magn Reson Med* **17**, 304 (1991).

- Ernst R.R., G. Bodenhausen, A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions", Oxford University Press, Oxford, 1987.
- Esau K., Ontogeny and structure of collenchyma and of vascular tissues in celery petioles. *Hilgardia* **10**, 431 (1936).
- Fenrich F.R.E., P.S. Allen, Transverse relaxation rate distributions for water protons in myelinated and unmyelinated nerves. *In* Book of Abstracts, 10th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 693 (1991).
- Fenrich F.R.E., "A Simulation and Experimental Study of Water Proton Relaxation in a White Matter Model", M.Sc. thesis, University of Alberta, 1992.
- Finch E.D., J.F. Harmon, B.H. Muller, Pulsed NMR measurements of the diffusion coefficient of water in muscle. *Arch Biochem Biophys* **147**, 299 (1971).
- Finelli D.A., A.L. Hopkins, W.R. Selman, R.C. Crumrine, S.U. Bhatti, W.D. Lust, Evaluation of experimental early acute cerebral ischemia before the development of edema: use of dynamic, contrast-enhanced and diffusion-weighted MR scanning. *Magn Reson Med* **27**, 189 (1992).
- Finelli D.A., G.C. Hurst, R.P. Gullapali, E.M. Bellon, Improved contrast of enhancing brain lesions in postgadolinium, T₁-weighted spin-echo images with use of magnetization transfer. *Radiology* **190**, 553 (1994).
- Ford J.C., D.B. Hackney, D.C. Aslop, H. Jara, P.M. Joseph, C.M. Hand, P. Black, MRI characterization of diffusion coefficients in a rat spinal cord injury model. *Magn Reson Med* **31**, 488 (1994).
- Forsen S., R.A. Hoffman, Study of moderately rapid chemical exchange reactions by means of nuclear magnetic resonance. *J Chem Phys* **39**, 2892 (1963).
- Forti A., G.M. Bydder, J.V. Hajnal, I.R. Young, Use of diffusion weighted magnetic resonance imaging in multiple sclerosis. *In* Book of Abstracts, 10th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 86 (1991).
- Fralix T.A., T.L. Ceckler, S.D. Wolff, S.A. Simon, R.S. Balaban, Lipid bilayer and water proton magnetization transfer: effect of cholesterol. *Magn Reson Med* **18**, 214 (1991).
- Franks F. (Ed.), "Water a Comprehensive Treatise", Vol 1-6, Plenum Press, New York, 1972.
- Fukushima E., S.B.W. Roeder, "Experimental Pulse NMR - A Nuts and Bolts Approach", Addison-Wesley Publishing Co., London, 1981a.
- Fukushima E., S.B.W. Roeder, "Experimental Pulse NMR - A Nuts and Bolts Approach", pp. 125-185, Addison-Wesley Publishing Co., London, 1981b.

- Garrido L., V.J. Wedeen, K.K. Kwong, U.M. Spencer, H.L. Kantor, Anisotropy of water diffusion in the myocardium of the rat. *Circ Res* **74**, 789 (1994).
- Gass A., G.J. Barker, D. Kidd, J.W. Thorpe, D. MacManus, A. Brennan, P.S. Tofts, A.J. Thompson, W.I. McDonald, D.H. Miller, Correlation of magnetization transfer ratios with clinical disability in multiple sclerosis. *Ann Neurol* **36**, 62 (1994).
- Gates L., I. Cameron, Restricted diffusion in human white matter for long diffusion times. *In Book of Abstracts, 2nd Annual Meeting of the Society of Magnetic Resonance, San Francisco*, p. 1037 (1994).
- Goldberg I.E., H.J. Aronen, K. Kwong, F.H. Hochberg, F. Pardo, R. McKinstry, T.J. Brady, B.R. Rosen, Correlation between spin-spin relaxation and diffusion coefficient in human central nervous system. *In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin*, p. 3710 (1992).
- Grad J., R.G. Bryant, Nuclear magnetic cross-relaxation spectroscopy. *J Magn Reson* **90**, 1 (1990).
- Grossman R.I., Magnetization transfer in multiple sclerosis. *Ann Neurol* **36**, S97 (1994).
- Hahn E.L., Spin echoes. *Phys Rev* **80**, 580 (1950).
- Hajnal J.V., C.J. Baudouin, A. Oatridge, I.R. Young, G.M. Bydder, Design and implementation of magnetization transfer pulse sequences for clinical use. *J Comput Assist Tomogr* **16**, 7 (1992a).
- Hajnal J.V., B. DeCoene, B.D. Lewis, C.J. Baudouin, F.M. Cowan, J.M. Penneck, I.R. Young, G.M. Bydder, High signal regions in normal white matter shown by heavily T₂-weighted CSF nulled IR sequences. *J Comput Assist Tomogr* **16**, 506 (1992b).
- Hanstock C.C., A.I. Faden, M.R. Bendall, R. Vink, Diffusion-weighted imaging differentiates ischemic tissue from traumatized tissue. *Stroke* **25**, 843 (1994).
- Hasegawa Y., L.L. Latour, C.H. Sotak, B.J. Dardzinski, M. Fisher, Temperature-dependent changes of apparent diffusion coefficient in normal and ischemic brain of rats. *J Cereb Blood Flow Metab* **14**, 383 (1994).
- Heide A.C., T.L. Richards, E.C. Alvord, J. Peterson, L.M. Rose, Diffusion imaging of experimental allergic encephalomyelitis. *Magn Reson Med* **29**, 478 (1993).
- Helpert J.A., R.J. Ordidge, R.A. Knight, The effect of cell membrane water permeability on the apparent diffusion coefficient of water. *In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin*, p. 1201 (1992).

- Henkelman R.M., X. Huang, Q.S. Xiang, G.J. Stanisz, S.D. Swanson, M.J. Bronskill, Quantitative interpretation of magnetization transfer. *Magn Reson Med* **29**, 759 (1993).
- Henkelman R.M., G.J. Stanisz, J.K. Kim, M.J. Bronskill, Anisotropy of NMR properties of tissues. *Magn Reson Med* **32**, 592 (1994).
- Hiehle J.F., R.E. Lenkinski, R.I. Grossman, V. Dousset, K.N. Ramer, M.D. Schnall, J.A. Cohen, F. Gonzalez-Scarano, Correlation of spectroscopy and magnetization transfer imaging in the evaluation of demyelinating lesions and normal appearing white matter in multiple sclerosis. *Magn Reson Med* **32**, 285 (1994).
- Hirokawa N., Molecular architecture and dynamics of the neuronal cytoskeleton, In "The Neuronal Cytoskeleton" (R.D. Burgoyne, Ed.), p. 25, Wiley-Liss, New York, 1991.
- Hodgkin A.L., A.F. Huxley, Action potentials recorded from inside a nerve fiber. *Nature* **144**, 710 (1939).
- Hodgkin A.L., R.D. Keynes, Experiments on the injection of substances into the squid giant axon by means of a microsyringe. *J Physiol* **131**, 592 (1956).
- Hoffman R.A., S. Forsen, Transient and steady-state Overhauser experiments in the investigation of relaxation processes. Analogies between chemical exchange and relaxation. *J Chem Phys* **45**, 2049 (1966).
- Holt R.W., J.L. duerk, J. Hua, G.C. Hurst, Estimation of Bloch model MT spin system parameters from z-spectral data. *Magn Reson Med* **31**, 122 (1994).
- Hong X., W.T. Dixon, Measuring diffusion in inhomogeneous systems in imaging mode using antisymmetric sensitizing gradients. *J Magn Reson* **99**, 561 (1992).
- Hore P.J., Solvent suppression in Fourier transform nuclear magnetic resonance. *J Magn Reson* **55**, 283 (1983).
- Horsfield M.A., G.J. Barker, W.I. McDonald, Self-diffusion in CNS tissue by volume-selective proton NMR. *Magn Reson Med* **31**, 637 (1994).
- Hossmann K.A., M. Fischer, K. Bockhorst, M. Hoehn-Berlage, NMR imaging of the apparent diffusion coefficient (ADC) for the evaluation of metabolic suppression and recovery after prolonged cerebral ischemia. *J Cereb Blood Flow Metab* **14**, 723 (1994).
- Howe F.A., A.G. Filler, B.A. Bell, J.R. Griffiths, Magnetic resonance neurography. *Magn Reson Med* **28**, 328 (1992).
- Hrovat M.I., C.G. Wade, NMR pulsed-gradient diffusion measurements. I. Spin-echo stability and gradient calibration. *J Magn Reson* **44**, 62 (1981a).

- Hrovat M.I., C.G. Wade, NMR pulsed-gradient diffusion measurements. II. Residual gradients and lineshape distortions. *J Magn Reson* **45**, 67 (1981b).
- Hu B.S., S.M. Conolly, G.A. Wright, D.G. Nishimura, A. Macovski, Pulsed saturation transfer contrast. *Magn Reson Med* **26**, 231 (1992).
- Hua J., G.C. Hurst, Analysis of on- and off-resonance magnetization transfer techniques. *J Magn Reson Imag* **5**, 113 (1995).
- Hürlimann M.D., K.G. Helmer, T.M. de Swiet, P.N. Sen, C.H. Sotak, Spin echoes in a constant gradient and in the presence of a simple restriction. *J Magn Reson A* **113**, 260 (1995).
- Jardetzky O., G.C.K. Roberts, "NMR in Molecular Biology", Academic Press, Orlando, 1981a.
- Jardetzky O., G.C.K. Roberts, "NMR in Molecular Biology", pp. 52-68, Academic Press, Orlando, 1981b.
- Jiang Q., M. Chopp, Z.G. Zhang, J.A. Helpert, R.J. Ordidge, J. Ewing, P. Jiang, B.A. Marchese, The effect of hypothermia on transient focal ischemia in rat brain evaluated by diffusion- and perfusion-weighted NMR imaging. *J Cereb Blood Flow Metab* **14**, 732 (1994).
- Karger J., H. Pfeifer, W. Heink, Principles and application of self-diffusion measurements by nuclear magnetic resonance, in "Advances in Magnetic Resonance, Vol. 12" (J.S. Waugh, Ed.), p. 1, Academic Press, San Diego, 1988a.
- Karger J., H. Pfeifer, W. Heink, Principles and application of self-diffusion measurements by nuclear magnetic resonance, in "Advances in Magnetic Resonance, Vol. 12" (J.S. Waugh, Ed.), p. 43, 51, Academic Press, San Diego, 1988b.
- Karlicek R.F., I.J. Lowe, A modified pulsed gradient technique for measuring diffusion in the presence of large background gradients. *J Magn Reson* **37**, 75 (1980).
- Keynes R.D., The nerve impulse and the squid. *Sci Am* **199**, 83 (1958).
- King M.D., J. Houseman, S.A. Roussel, N. van Bruggen, S.R. Williams, D.G. Gadian, q-space imaging of the brain. *Magn Reson Med* **32**, 707 (1994).
- Knight R.A., R.J. Ordidge, J.A. Helpert, M. Chopp, L.C. Rodolski, D. Peck, Temporal evolution of ischemic damage in rat brain measured by proton nuclear magnetic resonance imaging. *Stroke* **22**, 802 (1991).
- Knight R.A., M.O. Dereski, J.A. Helpert, R.J. Ordidge, M. Chopp, Magnetic resonance imaging assessment of evolving focal cerebral ischemia. Comparison with histopathology in rats. *Stroke* **25**, 1252 (1994).

- Koenig S.H., R.D. Brown III, M. Spiller, N. Lundbom, Relaxometry of brain: Why white matter appears bright in MRI, *Magn Reson Med* **14**, 482 (1990).
- Koenig S.H., Cholesterol of myelin is the determinant of gray-white contrast in MRI of brain. *Magn Reson Med* **20**, 285 (1991).
- Kozniewska E., T.P.L. Roberts, M. Tsuura, J. Mintorovitch, M.E. Moseley, J. Kucharczyk, NG-nitro-L-arginine delays the development of brain injury during focal ischemia in rats. *Stroke* **26**, 282 (1995).
- Kreis R., C. Fusch, C. Buesch, In vivo characterization of three water compartments in human white matter using a single voxel technique with short TE. *In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1963* (1992).
- Kucharczyk J., J. Mintorovitch, M.E. Moseley, H.S. Agari, R.J. Sevic, N. Derugin, D. Norman, Ischemic brain damage: reduction by sodium-calcium channel modulator RC-87476. *Radiology* **179**, 221 (1991).
- Kucharczyk W., P.M. MacDonald, G.J. Stanis, R.M. Henkelman, Magnetic resonance relaxivity and magnetization transfer of white matter lipids: the importance of cerebroside and pH. *Radiology* **192**, 521 (1994).
- Kurki T., M. Komu, Spin-lattice relaxation and magnetization transfer in intracranial tumors in vivo: effects of Gd-DTPA on relaxation parameters. *Magn Reson Imaging* **13**, 379 (1995).
- Kushmerick M.J., R.J. Podolsky, Ionic mobility in cells. *Science* **166**, 1297 (1969).
- Kuwata K., D. Brooks, H. Yang, T. Schleich, Relaxation-matrix formalism for rotating-frame spin-lattice proton NMR relaxation and magnetization transfer in the presence of an off-resonance-irradiation field. *J Magn Reson B* **104**, 11 (1994).
- Kwong K.K., Y.C. Chen, T.L. Davis, B.R. Rosen, Multiexponential diffusion signal decays in human brain at large b values. *In Book of Abstracts, 1st Annual Meeting of the Society of Magnetic Resonance, Dallas, p. 545* (1994).
- Laicher G., D.C. Ailion, A.G. Cutillo, Water self diffusion in excised rat lung. *In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1203* (1992).
- Larsson H.B.W., P. Christiansen, C. Thomsen, M. Stubgaard, J. Frederiksen, O. Henriksen, In vivo measurement of water self diffusion in patients with multiple sclerosis. *In Book of Abstracts, 9th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 150* (1990).
- Larsson H.B.W., C. Thomsen, J. Frederiksen, M. Stungard, O. Henriksen, In vivo magnetic resonance diffusion measurement in the brain of patients with multiple sclerosis. *Magn Reson Imaging* **10**, 7 (1992).

- Latour L.L., K. Svoboda, P.P. Mitra, C.H. Sotak, Time-dependent diffusion of water in a biological model system. *Proc Natl Acad Sci USA* **91**, 1229 (1994a).
- Latour L.L., Y. Hasegawa, J.E. Formato, M. Fisher, C.H. Sotak, Spreading waves of decreased diffusion coefficient after cortical stimulation in the rat brain. *Magn Reson Med* **32**, 189 (1994b).
- Lauger P., L. Packer, V. Vasilescu (Eds.), "Water and Ions in Biological Systems", Birkhauser-Verlag, Basel, 1988.
- Le Bihan D., E. Breton, D. Lallemand, P. Grenier, E. Cabanis, M. Laval-Jeantet, MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology* **161**, 401 (1986).
- Le Bihan D., Molecular diffusion in nuclear magnetic resonance imaging. *Magn Reson Quart* **7**, 1 (1991).
- Le Bihan D., R. Turner, P. Douek, N. Patronas, Diffusion MR imaging: clinical applications. *AJR* **159**, 591 (1992).
- Le Bihan D., R. Turner, P. Douek, Is water diffusion restricted in human brain white matter? An echo-planar NMR imaging study. *NeuroReport* **4**, 887 (1993).
- Lexa F.J., R.I. Grossman, A.C. Rosenquist, MR of Wallerian degeneration in the feline visual system: characterization by magnetization transfer rate with histopathologic correlation. *AJNR* **15**, 201 (1994).
- Lian J., D.S. Williams, I.J. Lowe, Magnetic resonance imaging of diffusion in the presence of background gradients and imaging of background gradients. *J Magn Reson A* **106**, 65 (1994).
- Lo E.H., K. Matsumoto, A.R. Pierce, L. Garrido, D. Luttinger, Pharmacologic reversal of acute changes in diffusion-weighted magnetic resonance imaging in focal cerebral ischemia. *J Cereb Blood Flow Metab* **14**, 597 (1994).
- Luck W.A.P., Forward, In "Structure of Water and Aqueous Solutions" (W.A.P. Luck, Ed.), p VI, Verlag Chemie, Germany, 1974.
- Lundbom N., Determination of magnetization transfer contrast in tissue: an MR imaging study of brain tumours. *AJR* **159**, 1279 (1992).
- MacFall J.R., H. Beneviste, J. Maki, G.A. Johnson, L. Hedlund, G. Cofer, Restricted diffusion measurements in sub-acute rat brain. In Book of Abstracts, 9th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 1119 (1990).
- MacKay A., K. Whittall, J. Adler, D. Li, D. Paty, D. Graeb, In vivo visualization of myelin water in brain by magnetic resonance. *Magn Reson Med* **31**, 673 (1994).

- Mann B.E., The application of the Forsen-Hoffman spin-saturation method of measuring rates of exchange to the ^{13}C NMR spectrum of N,N-dimethylformamide. *J Magn Reson* **25**, 91 (1977).
- Mansfield P., Multi-planar image formation using NMR spin-echoes. *J Phys C* **10**, L55 (1977).
- Mansfield P., P.G. Morris, "NMR Imaging in Biomedicine", pp. 271-275, Academic Press, New York, 1982a.
- Mansfield P., P.G. Morris, "NMR Imaging in Biomedicine", pp. 266-267, Academic Press, New York, 1982b.
- McConnell H.M., Reaction rates by nuclear magnetic resonance. *J Chem Phys* **28**, 430 (1958).
- McConnell J., "The Theory of Nuclear Magnetic Relaxation in Liquids", Cambridge University Press, Cambridge, 1987.
- McGowan J.C., M.D. Schnall, J.S. Leigh, Magnetization transfer imaging with pulsed off-resonance saturation: variation in contrast with saturation duty cycle. *J Magn Reson Imag* **4**, 79 (1994).
- Meiboom S., D. Gill, Modified spin-echo method for measuring nuclear relaxation times. *Rev Sci Instrum* **29**, 688 (1958).
- Menon R.S., "Some Mechanisms of Water Proton NMR Relaxation in Model Tissue Systems", Ph.D. thesis, University of Alberta, 1990.
- Menon R.S., P.S. Allen, Application of continuous relaxation time distributions to the fitting of data from model systems and excised tissue. *Magn Reson Med* **20**, 214 (1991).
- Menon R.S., M.S. Rusinko, P.S. Allen, Proton relaxation studies of water compartmentalization in a model neurological system. *Magn Reson Med* **28**, 264 (1992).
- Merboldt K.D., W. Hanicke, J. Frahm, Self-diffusion NMR imaging using stimulated echoes. *J Magn Reson* **64**, 479 (1985).
- Merboldt K.D., D. Horstmann, W. Hanicke, H. Bruhn, J. Frahm, Molecular self-diffusion of intracellular metabolites in rat brain in vivo investigated by localized proton NMR diffusion spectroscopy. *Magn Reson Med* **29**, 125 (1993).
- Merboldt K.D., W. Hanicke, H. Bruhn, M.L. Gyngell, J. Frahm, Diffusion imaging of the human brain in vivo using high-speed STEAM MRI. *Magn Reson Med* **23**, 179 (1992).
- Mikulis D., D. Chien, K. Kwong, R. Buxton, K. Davis, T. Brady, J. Pile-Spellman, Diffusion magnetic resonance imaging in multiple sclerosis. *In Book of*

Abstracts, 7th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 762 (1988).

- Mills R., Self-diffusion in normal and heavy water in the range 1-45 °. *J Phys Chem* **77**, 685 (1973).
- Minematsu K., L. Li, M. Fisher, C.H. Sotak, M.A. Davis, M.S. Fiandaca, Diffusion-weighted magnetic resonance imaging: rapid and quantitative detection of focal brain ischemia. *Neurology* **42**, 235 (1992a).
- Minematsu K., L. Li, C.H. Sotak, M.A. Davis, M. Fisher, Reversible focal ischemic injury demonstrated by diffusion-weighted magnetic resonance imaging in rats. *Stroke* **23**, 1304 (1992b).
- Minematsu K., M. Fisher, L. Li, M.A. Davis, A.G. Knapp, R.E. Cotter, R.N. McBurney, C.H. Sotak, Effects of a novel NMDA antagonist on experimental stroke rapidly and quantitatively assessed by diffusion-weighted MRI. *Neurology* **43**, 397 (1993).
- Mintorovitch J., M.E. Moseley, L. Chileuitt, H. Shimizu, Y. Cohen, P.R. Weinstein, Comparison of diffusion- and T₂-weighted MRI for the early detection of cerebral ischemia and reperfusion in rats. *Magn Reson Med* **18**, 39 (1991).
- Mintorovitch J., G.Y. Yang, H. Shimizu, J. Kucharczyk, P.H. Chan, P.R. Weinstein, Diffusion-weighted magnetic resonance imaging of acute focal cerebral ischemia: comparison of signal intensity with changes in brain water and Na⁺, K⁺-ATPase activity. *J Cereb Blood Flow Metab* **14**, 332 (1994).
- Moonen C.T.W., J. Pekar, M.H.M. de Vleeschouwer, P. van Gelderen, P.C.M. van Zijl, D. DesPres, Restricted and anisotropic displacement of water in healthy cat brain and in stroke studied by NMR diffusion imaging. *Magn Reson Med* **19**, 327 (1991).
- Mori S., P.C.M. van Zijl, Diffusion weighting by the trace of the diffusion tensor within a single scan. *Magn Reson Med* **33**, 41 (1995).
- Morrison C., G. Stanisz, R.M. Henkelman, A model for magnetization transfer in tissues. *Magn Reson Med* **33**, 475 (1995a).
- Morrison C., R.M. Henkelman, Modelling magnetization transfer in biological-like systems using a semi-solid pool with a super-Lorentzian lineshape and dipolar reservoir. *J Magn Reson B* in press (1995b).
- Moseley M.E., Y. Cohen, J. Mintorovitch, L. Chileuitt, H. Shimizu, J. Tsuruda, D. Norman, P. Weinstein, Evidence of anisotropic self-diffusion in cat brain. In Book of Abstracts, 8th Annual Meeting of the Society of Magnetic Resonance in Medicine, Amsterdam, p. 136 (1989).
- Moseley M.E., Y. Cohen, J. Kucharczyk, J. Mintorovitch, H.S. Agari, M.F. Wendland, J. Tsuruda, D. Norman, Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. *Radiology* **176**, 439 (1990a).

- Moseley M.E., Y. Cohen, J. Mintorovitch, L. Chileuitt, H. Shimizu, J. Kucharczyk, M.F. Wendland, P.R. Weinstein, Early detection of regional cerebral ischemia in cats: comparison of diffusion- and T₂-weighted MRI and spectroscopy. *Magn Reson Med* **14**, 330 (1990b).
- Moseley M.E., J. Kucharczyk, J. Mintorovitch, Y. Cohen, J. Kurhanewicz, W. Derugin, H. Asgari, D. Norman, Diffusion-weighted MR imaging of acute stroke: correlation with T₂-weighting and magnetic susceptibility-enhanced MR imaging in cats. *AJNR* **11**, 423 (1990c).
- Moseley M.E., M.F. Wendland, J. Kucharczyk, Magnetic resonance imaging of diffusion and perfusion. *Top Magn Reson Imaging* **3**, 50 (1991a).
- Moseley M.E., M.F. Wendland, Acute effects of exercise on echo-planar T₂- and diffusion-weighted MRI of skeletal muscle in volunteers. *In* Book of Abstracts, 10th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, p. 108 (1991b).
- Moseley M.E., A. de Crespigny, W.M. Chew, Diffusion/perfusion magnetic resonance imaging. *Neuroimaging Clinics of North America* **2**, 693 (1992).
- Muller R.N., M.J. Marsh, M.L. Bernardo, P.C. Lauterbur, True 3-D imaging of limbs by NMR zeugmatography with off-resonance irradiation. *Europ J Radiol* **3**, 286 (1983).
- Murday J.S., Measurement of magnetic field gradient by its effect on the NMR free induction decay. *J Magn Reson* **10**, 111 (1973).
- Nathaniel E.J.H., D.C. Pease, Degenerative changes in rat dorsal roots during Wallerian degeneration. *J Ultrastruct Res* **9**, 511 (1963).
- Nicholls J.G., A.R. Martin, B.G. Wallace, *In* "From Neuron to Brain: A Cellular and Molecular Approach to the Function of the Nervous System," 3rd Ed., pp. 138-139, Sinauer Associates, Sunderland, MA, 1992a.
- Nicholls J.G., A.R. Martin, B.G. Wallace, *In* "From Neuron to Brain: A Cellular and Molecular Approach to the Function of the Nervous System," 3rd Ed., pp. 287-291, Sinauer Associates, Sunderland, MA, 1992b.
- Nicholson C.A., Quantitative analysis of extracellular space using the method of TMA⁺ iontophoresis and the issue of TMA⁺ uptake. *Canadian Journal of Physiology and Pharmacology* **70**, S314 (1992).
- Nicholson C., Diffusion of ions and macromolecules in the brain. *In* Functional MRI of the Brain Syllabus, Workshop Presented by the Society of Magnetic Resonance in Medicine and the Society for Magnetic Resonance Imaging, Arlington, VA, p. 1 (1993).
- Niendorf T., D.G. Norris, D. Leibfritz, Detection of apparent restricted diffusion in healthy rat brain at short diffusion times. *Magn Reson Med* **32**, 672 (1994).

- Noggle J.H., R.E. Schirmer, "The Nuclear Overhauser Effect: Chemical Applications", pp. 13-17, Academic Press, New York, 1971.
- Nomura Y., H. Sakuma, T. Tagami, K. Takeda, T. Nakagawa, Y. Tamagawa, H. Kimura, Y. Ishii, K. Torizuka, Diffusion anisotropy in adult and neonatal human brain - assessment with intravoxel incoherent motion (IVIM) imaging. *In* Book of Abstracts, 9th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 374 (1990).
- Oatridge A., J.V. Hajnal, F.M. Cowan, C.J. Baudouin, I.R. Young, G.M. Bydder, MRI diffusion-weighted imaging of the brain: contributions to image contrast from CSF signal reduction, use of a long echo time and diffusion effects. *Clinical Radiology* **47**, 82 (1993).
- Ordidge R.J., J.A. Helpert, R.A. Knight, Z. Qing, K.M.A. Welch, Investigation of cerebral ischemia using magnetization transfer contrast (MTC) MR imaging. *Magn Reson Imaging* **9**, 985 (1991).
- Ordidge R.J., J.A. Helpert, Z.X. Qing, R.A. Knight, V. Nagesh, Correction of motional artifacts in diffusion-weighted MR images using navigator echoes. *Magn Reson Imaging* **12**, 455 (1994).
- Paty D.W., D.K.B. Li, Neuroimaging in multiple sclerosis, *In* "Clinical Neuroimaging" (W.H. Theodore, Ed.), p. 249, Alan R. Liss Inc., New York, 1988.
- Pierpaoli C., A. Righini, I. Linfante, J.H. Tao-Cheng, J.R. Alger, G. Di Chiro, Histopathologic correlates of abnormal water diffusion in cerebral ischemia: diffusion-weighted MR imaging and light and electron microscopic study. *Radiology* **189**, 439 (1993).
- Pike G.B., B.S. Hu, G.H. Glover, D.R. Enzmann, Magnetization transfer time-of-flight magnetic resonance angiography. *Magn Reson Med* **25**, 372 (1992).
- Pike G.B., G.H. Glover, B.S. Hu, D.R. Enzmann, Pulsed magnetization transfer spin-echo imaging. *J Magn Reson Imag* **3**, 531 (1993).
- Prager J.M., J.D. Rosenblum, D.C. Huddle, M.J. Lipton, The magnetization transfer effect in cerebral infarcts. *In* "Proceedings of Workshop on Water/Macromolecule Magnetization Transfer in MRI", SMRM, p. 21 (1993).
- Price W.S., P.W. Kuchel, Effect of nonrectangular field gradient pulses in the Stejskal and Tanner (diffusion) pulse sequence. *J Magn Reson* **94**, 133 (1991).
- Purcell E.M., H.C. Torrey, R.V. Pound, Resonance absorption by nuclear magnetic resonance moments in a solid. *Phys Rev* **69**, 37 (1946).
- Rabi I.I., J.R. Zacharias, S. Millman, P. Kusch, A new method of measuring nuclear magnetic moments. *Phys Rev* **53**, 318 (1938).

- Rabi I.I., S. Millman, P. Kusch, J.R. Zacharias, The molecular beam resonance method for measuring nuclear magnetic moments. The magnetic moments of ${}^3\text{Li}^6$, ${}^3\text{Li}^7$, ${}^9\text{F}^{19}$. *Phys Rev* **55**, 526 (1939).
- Reeves L.W., Application of non-selective pulse NMR experiments - diffusion and chemical exchange, in "Dynamic Nuclear Magnetic Resonance Spectroscopy" (L.M. Jackman, F.A. Cotton, Eds.), pp. 83-105, Academic Press, New York, 1975.
- Rorschach H.E., D.C. Chang, C.F. Hazlewood, B.L. Nichols, The diffusion of water in striated muscle. *Ann NY Acad Sci* **204**, 444 (1973).
- Rubinson K.A., P.F. Baker, The flow properties of axoplasm in a defined chemical environment: influence of anions and calcium. *Proc Roy Soc Lond B* **205**, 323 (1979).
- Rutherford M.A., F.M. Cowan, A.Y. Manzur, L.M.S. Dubowitz, J.M. Pennock, J.V. Hajnal, I.R. Young, G.M. Bydder, MR imaging of anisotropically restricted diffusion in the brain of neonates and infants. *J Comput Assist Tomogr* **15**, 188 (1991).
- Sakuma H., Y. Nomura, K. Takeda, T. Tagami, T. Nakagawa, Y. Tamagawa, Y. Ishii, T. Tsukamoto, Adult and neonatal human brain: diffusional anisotropy and myelination with diffusion-weighted MR imaging. *Radiology* **180**, 229 (1991).
- Schlaepfer W.W., H. Hager, Ultrastructural studies of INH-induced neuropathy in rats. *Am J Pathol* **45**, 209 (1964).
- Schneider E., R.W. Prost, G.H. Glover, Pulsed magnetization transfer versus continuous wave irradiation for tissue contrast enhancement. *J Magn Reson Imag* **3**, 417 (1993).
- Schoeninger J.S., N. Aiken, E. Hsu, S.J. Blackband, Relaxation-time and diffusion NMR microscopy of single neurons. *J Magn Reson B* **103**, 261 (1994).
- Segawa F., K. Kamada, Y. Mizoi, N. Sunohara, K. Shimizu, Y. Hashimoto, Molecular diffusion anisotropy in peripheral nerve. In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1601 (1992a).
- Segawa F., K. Kamada, Y. Mizoi, N. Sunohara, K. Shimizu, Y. Hashimoto, Diffusion coefficient and blood barrier damage in multiple sclerosis. In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1706 (1992b).
- Segawa F., M. Kinoshita, J. Kishibayashi, K. Kamada, N. Sunohara, K. Shimizu, Y. Hashimoto, Diffusion images of Wallerian degeneration. In Book of Abstracts, 12th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, p. 595 (1993).

- Sevick R.J., F. Kanda, J. Mintorovitch, A.I. Arieff, J. Kucharczyk, J.S. Tsuruda, D. Norman, M.E. Moseley, Cytotoxic brain edema: assessment with diffusion-weighted MR imaging. *Radiology* **185**, 687 (1992).
- Schroder J.M., Degeneration and regeneration of myelinated nerve fibers in experimental neuropathies, In "Peripheral Neuropathy, Vol. 1" (P.J. Dyck, P.K. Thomas, and E.H. Lambert, Eds.), pp. 337-362, W.B. Saunders Company, Philadelphia, 1975.
- Slichter C.P., "Principles of Magnetic Resonance", Springer-Verlag, Berlin, 1990a.
- Slichter C.P., "Principles of Magnetic Resonance", pp. 12-13, 20-21, Springer-Verlag, Berlin, 1990b.
- Smith D.S., V. Jarlsfor, B.F. Cameron, Morphological evidence for the participation of microtubules in axonal transport. *Ann NY Acad Sci* **253**, 472 (1975).
- Solomon I., Relaxation processes in a system of two spins. *Phys Rev* **99**, 559 (1955).
- Sotak C.H., L.Li, MR imaging of anisotropic and restricted diffusion by simultaneous use of spin and stimulated echoes. *Magn Reson Med* **26**, 174 (1992).
- Stejskal E.O., J.E. Tanner, Spin diffusion measurements: spin-echoes in the presence of a time-dependent field gradient. *J Chem Phys* **42**, 288 (1965).
- Stewart W.A., A.L. MacKay, K.P. Whittall, G.R.W. Moore, D.W. Paty, Spin-spin relaxation in experimental allergic encephalomyelitis. Analysis of CPMG data using a non-linear least squares method and linear inverse theory. *Magn Reson Med* **29**, 767 (1993).
- Stilbs P., Fourier transform pulsed gradient spin-echo studies of molecular diffusion, in "Progress in NMR Spectroscopy, Vol. 19" (J.W. Emsley, J. Feeney, L.H. Sutcliffe, Eds.), p. 1, Pergamon Journals, Oxford, 1987.
- Sunderland S., Degeneration of the axon and associated changes, In "Nerve and Nerve Injuries", pp. 79-95, E and S Livingstone Ltd., Edinburgh, 1968.
- Szafer A., J. Zhong, J.C. Gore, Theoretical model for water diffusion in tissues. *Magn Reson Med* **33**, 697 (1995).
- Takahashi M., B. Fritz-Zieroth, T. Chikugo, H. Ogawa, Differentiation of chronic lesions after stroke in stroke-prone spontaneously hypertensive rats using diffusion weighted MRI. *Magn Reson Med* **30**, 485 (1993).
- Tanner J.E., Use of the stimulated echo in NMR diffusion studies. *J Chem Phys* **52**, 2523 (1970).
- Tanner J.E., Self diffusion of water in frog muscle. *Biophys J* **28**, 107 (1979).

- Taylor D.G., M.C. Bushell, The spatial mapping of translational diffusion coefficients by the NMR imaging technique. *Phys Med Biol* **30**, 345 (1985).
- Thomsen C., O. Henriksen, P. Ring, In vivo measurement of water self diffusion in the human brain by magnetic resonance imaging. *Acta Radiol* **28**, 353 (1987).
- Tien R.D., G.J. Felsberg, H. Friedman, M. Brown, J. MacFall, MR imaging of high-grade cerebral gliomas: value of diffusion-weighted echoplanar pulse sequences. *AJR* **162**, 671 (1994).
- Tomiak M.M., J.D. Rosenblum, J.M. Prager, C.E. Metz, Magnetization transfer: a potential method to determine the age of multiple sclerosis lesions. *AJNR* **15**, 1569 (1994).
- Torrey H.C., Bloch equations with diffusion terms. *Phys Rev* **104**, 563 (1956).
- Trappeniers N.J., C.J. Gerritsma, P.H. Oosting, The self diffusion coefficient of water, at 25 °C, by means of spin-echo technique. *Phys Lett* **18**, 256 (1965).
- Trudeau J.D., J. Hawkins, W.T. Dixon, Separating effects of susceptibility induced gradients from restricted diffusion on the ADC. In Book of Abstracts, 2nd Annual Meeting of the Society of Magnetic Resonance, San Francisco, p. 1050 (1994).
- Tsuruda J.S., W.M. Chew, M.E. Moseley, D. Norman, Diffusion-weighted MR imaging of the brain: value of differentiating between extraaxial cysts and epidermoid tumours. *AJNR* **11**, 925 (1990).
- Turner R., D. Le Bihan, Single-shot diffusion imaging at 2.0 tesla. *J Magn Reson* **86**, 445 (1990a).
- Turner R., D. Le Bihan, J. Maier, R. Vavrek, L.K. Hedges, J. Pekar, Echo-planar imaging of intravoxel incoherent motions. *Radiology* **177**, 407 (1990b).
- van Dusschoten D., C.T.W. Moonen, H. van As, P.A. de Jager, Combining pulsed field gradient NMR and Carr Purcell imaging to discriminate multiple diffusion constants. In Book of Abstracts, 11th Annual Meeting of the Society of Magnetic Resonance in Medicine, Berlin, p. 1208 (1992).
- van Gelderen P., D. DesPres, P.C.M. van Zijl, C.T.W. Moonen, Evaluation of restricted diffusion in cylinders. Phosphocreatine in rabbit leg muscle. *J Magn Reson B* **103**, 255 (1994a).
- van Gelderen P., M.H.M. de Vleeschouwer, D. DesPres, J. Pekar, P.C.M. van Zijl, C.T.W. Moonen, Water diffusion and acute stroke. *Magn Reson Med* **31**, 154 (1994b).
- van Zijl P.C.M., C.T.W. Moonen, P. Faustino, J. Pekar, O. Kaplan, J.S. Cohen, Complete separation of intracellular and extracellular information in NMR spectra of perfused cells by diffusion-weighted spectroscopy. *Proc Natl Acad Sci (USA)* **88**, 3228 (1991).

- Vasilescu V., E. Katona, V. Simplaceanu, D. Demco, Water compartments in the myelinated nerve. III. Pulsed NMR results. *Experientia* **34**, 1443 (1978).
- Warach S., D. Chien, W. Li, M. Ronthal, R.R. Edelman, Fast magnetic resonance diffusion-weighted imaging of acute human stroke. *Neurology* **42**, 1717 (1992).
- Warach S., J. Gaa, B. Siewert, P. Wielopolski, R.R. Edelman, Acute human stroke studied by whole brain echo planar diffusion-weighted magnetic resonance imaging. *Ann Neurol* **37**, 231 (1995).
- Wesbey G.E., M.E. Moseley, R.L. Ehman, Translational molecular self-diffusion in magnetic resonance imaging: effects and applications. *Invest Radiol* **19**, 491 (1984).
- Whittall K.P., A.L. MacKay, Quantitative interpretation of NMR relaxation data. *J Magn Reson* **84**, 134 (1989).
- Williams W.D., E.F.W. Seymour, R.M. Cotts, A pulsed-gradient multiple spin-echo NMR technique for measuring diffusion in the presence of background magnetic field gradients. *J Magn Reson* **31**, 271 (1978).
- Wimberger D.M., T.P. Roberts, A.J. Barkovich, L.M. Prayer, M.E. Moseley, J. Kucharczyk, Identification of "premyelination" by diffusion-weighted MRI. *J Comput Assist Tomogr* **19**, 28 (1995).
- Woessner D.E., B.S. Snowden, Pulsed NMR study of water in agar gels. *J Colloid and Interface Sci* **34**, 290 (1970).
- Wolff S.D., R.S. Balaban, Magnetization transfer contrast (MTC) and water proton relaxation in vivo. *Magn Reson Med* **10**, 135 (1989).
- Wu X., Lineshape of magnetization transfer via cross relaxation. *J Magn Reson* **94**, 186 (1991).
- Yeung H.N., A.M. Eisen, Magnetization transfer contrast with periodic pulsed saturation. *Radiology* **183**, 209 (1992a).
- Yeung H.N., S.D. Swanson, Transient decay of longitudinal magnetization in heterogeneous spin systems under selective saturation. *J Magn Reson* **99**, 466 (1992b).
- Yeung H.N., R.S. Adler, S.D. Swanson, Transient decay of longitudinal magnetization in heterogeneous spin systems under selective saturation. IV. Reformulation of the spin-bath model equations by the Redfield-Provotorov theory. *J Magn Reson A* **106**, 37 (1994).
- Young J.Z., The structure of nerve fibers in cephalopods and crustacea. *Proc Roy Soc B* **121**, 319 (1936).

- Young J.Z., The functioning of the giant nerve fibers of the squid. *J Exp Biol* **15**, 170 (1938).
- Zhong J., J.C. Gore, Studies of restricted diffusion in heterogeneous media containing variations in susceptibility. *Magn Reson Med* **19**, 276 (1991a).
- Zhong J., R.P. Kennan, J.C. Gore, Effects of susceptibility variations in NMR measurements of diffusion. *J Magn Reson* **95**, 267 (1991b).
- Zhong J., O.A.C. Petroff, J.W. Prichard, J.C. Gore, Barbiturate-reversible reduction of water diffusion coefficient in flurothyl-induced status epilepticus. *Magn Reson Med* **33**, 253 (1995).

APPENDIX 1

Schematic Diagrams of the Gradient Coils

The schematic diagrams and the construction method for the gradient coils that were used in our measurements of diffusion are presented in this appendix. The gradient coils were designed and constructed by DDB (Damji Doran Beaulieu) Incorporated®. The minimum dimensions of the current paths were dictated by the region of linearity required for our samples (i.e. ± 1 cm about the origin). Given a maximum current of 30 A and the minimum dimensions of the gradient coils, 25 and 36 turns were chosen to get sufficient gradient strengths for the z and x gradients, respectively. The maximum resistance of the total length of wires for each gradient was chosen to be 0.8Ω in order to match the resistance of the original Bruker gradient coils. Therefore, #24 AWG (0.511 mm diameter) and #17 AWG (1.15 mm diameter) wires were chosen for the z and x gradients.

A very rigid material (G7) was used for the former to ensure that the gradients had sufficient stability and vigor. The gradient construction involved the machining of five components, a central component for the z gradient and four quadrants for the x gradient (Fig. A1.1). The interlocking pattern between the central component and the four quadrants was incorporated into the design to minimize any potential vibrations from forces that act on the quadrants when current is passed through the x gradient. The 3 cm diameter bore throughout the central component gave sufficient room for an rf coil. Also, a 1 cm diameter hole at the top of the gradient set provided an entry portal for the samples. Careful attention was paid to the dimensions of the grooves for the wires. The depths of the grooves were chosen to ensure that the centers of the 5x5 and 6x6 squares of wires were at the calculated current positions since the theory assumes that the currents are point sources. The widths of the grooves were designed so that the wires would be very snug (i.e. no room for vibration). However, a small amount of tolerance is required if the wires are to fit into the grooves. An additional groove was required to provide an entry/return path to connect the windings of the z gradient to the connector at the back of the gradient set. A similar groove was required for the x gradient. Furthermore, extra grooves and spaces were necessary for connecting wires between the individual quadrants. The dimensions of the five

constituent parts of the gradient former are illustrated in a series of diagrams (Fig. A1.2).

The wires were wound tightly one layer at a time about the individual formers. The wires often had to be gently hammered into place. Obviously, it is very important to make sure that the wires are wound in the appropriate directions (Fig. A1.3 for the more complex x gradient). Proper pre-labelling of the wires helps to ensure which wires are the entry wires and which are the return wires for each quadrant of the x gradient. The z gradient is wound with one continuous piece of wire and hence has less difficulties associated with its construction. Once the 5 or 6 layers of wires had been wound and visually inspected for tautness and integrity (i.e. no noticeable chips in the wire insulation), PC-7 epoxy bonding agent was used to secure the wires. Once the epoxy had hardened, the four quadrants of the x gradient were slid onto the central former containing the z gradient. The wires connecting the individual quadrants were soldered and were secured in place with epoxy. A connector was attached to the back of the gradient set to provide an interface to the Bruker gradient drivers. In addition to the z and x gradients of the new gradient set, the y gradient of the Bruker gradient set also had to be connected to the gradient drivers in order to provide us with sufficient first order linear gradients to shim on the sample.

The completed gradient coils were secured to a heavy plexiglass tray that could be inserted into the bore of the magnet. This allowed reproducible placement of the gradients and ensured that the sample was in the isocenter of the magnet. The tray was also taped to the front face of the magnet to prevent any rotation of the entire assembly.

My only suggestion for improvement of the gradient coils would be to use a thicker diameter wire with better insulation for the z gradient. The #24 AWG wire has proven to be more susceptible to thermal breakdown.

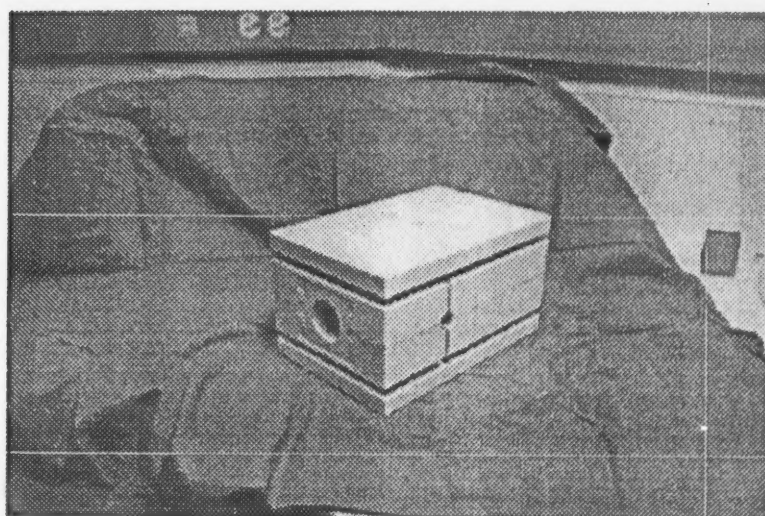
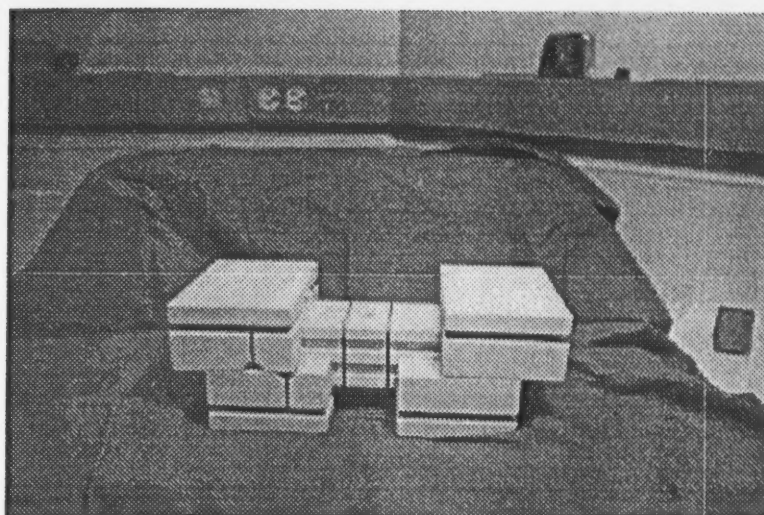


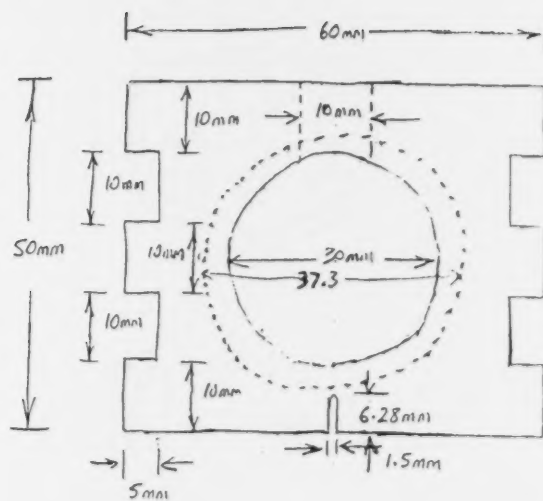
Figure A1.1 Photographs of the gradient former components.

Figure A1.2 The schematic diagrams of the gradient coils that were used for our measurements of diffusion are presented in the following pages (drawings are by K. Damji, NMR Group).

Rear view

1 of 4

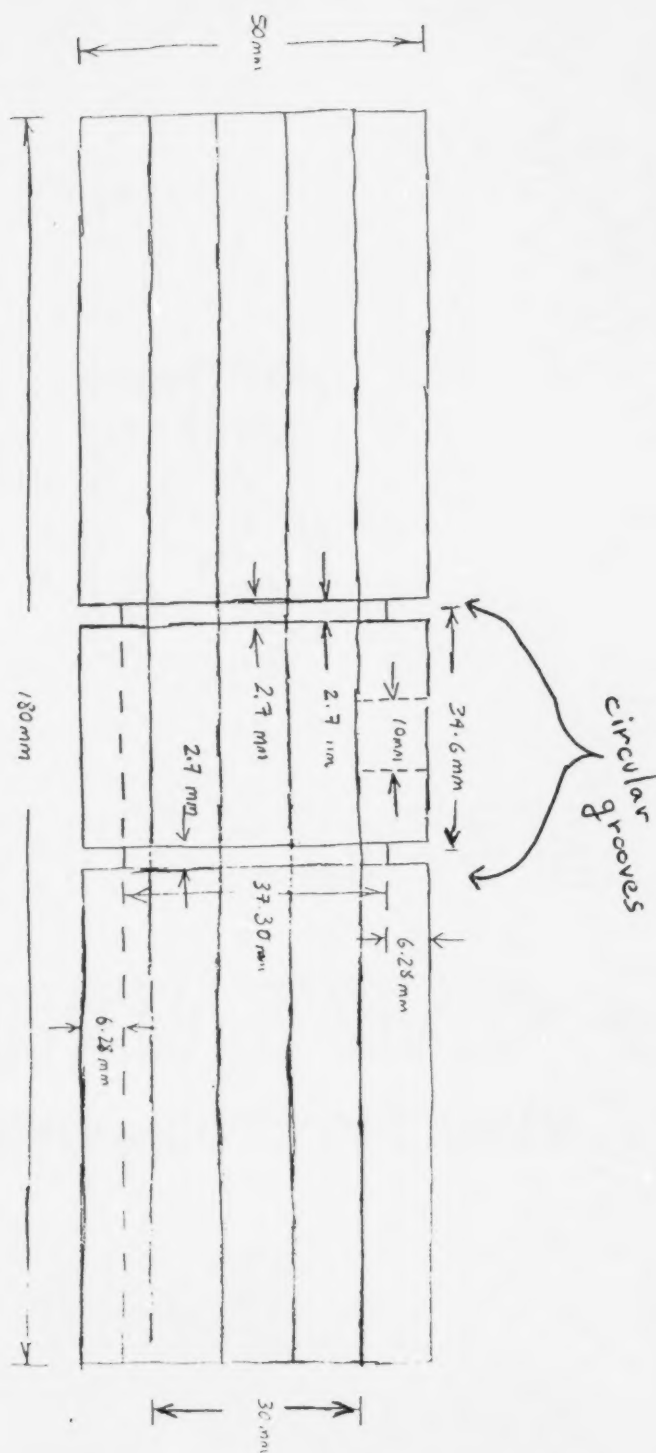
z-gradient

Rear View:

Side view

2 of 4

z-gradient

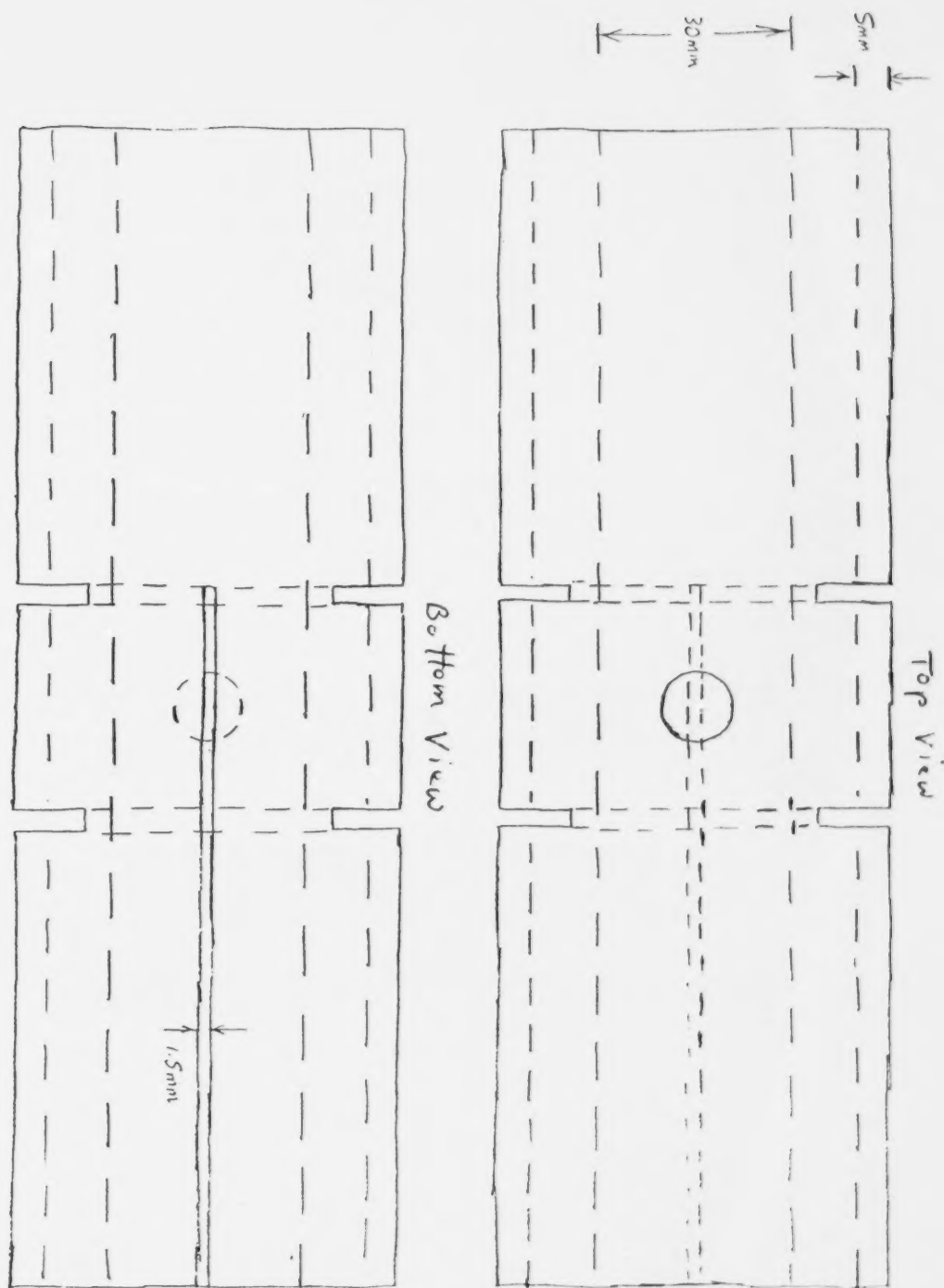


above 2.7 mm instead of 2.56 mm

Top and bottom views

3 of 4

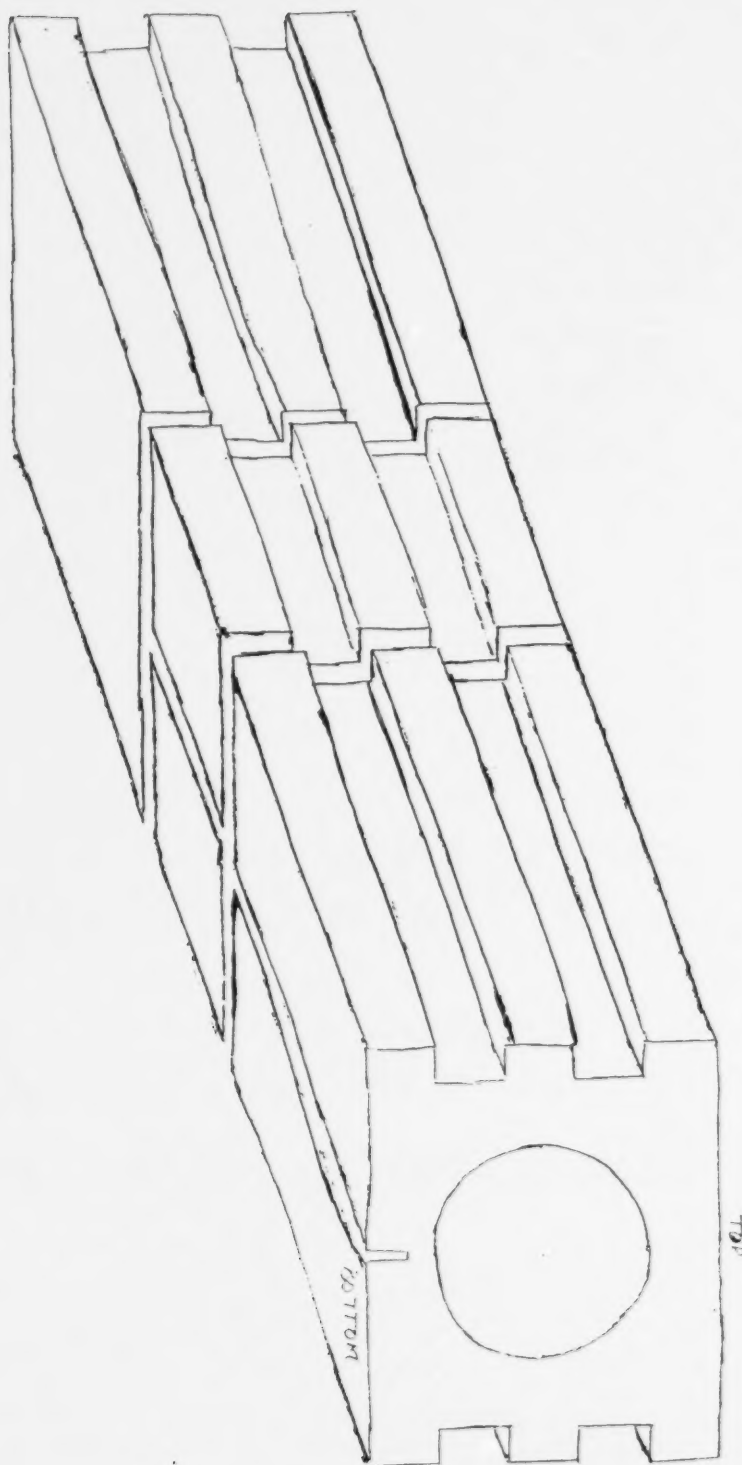
z-gradient



3D view

4 of 4

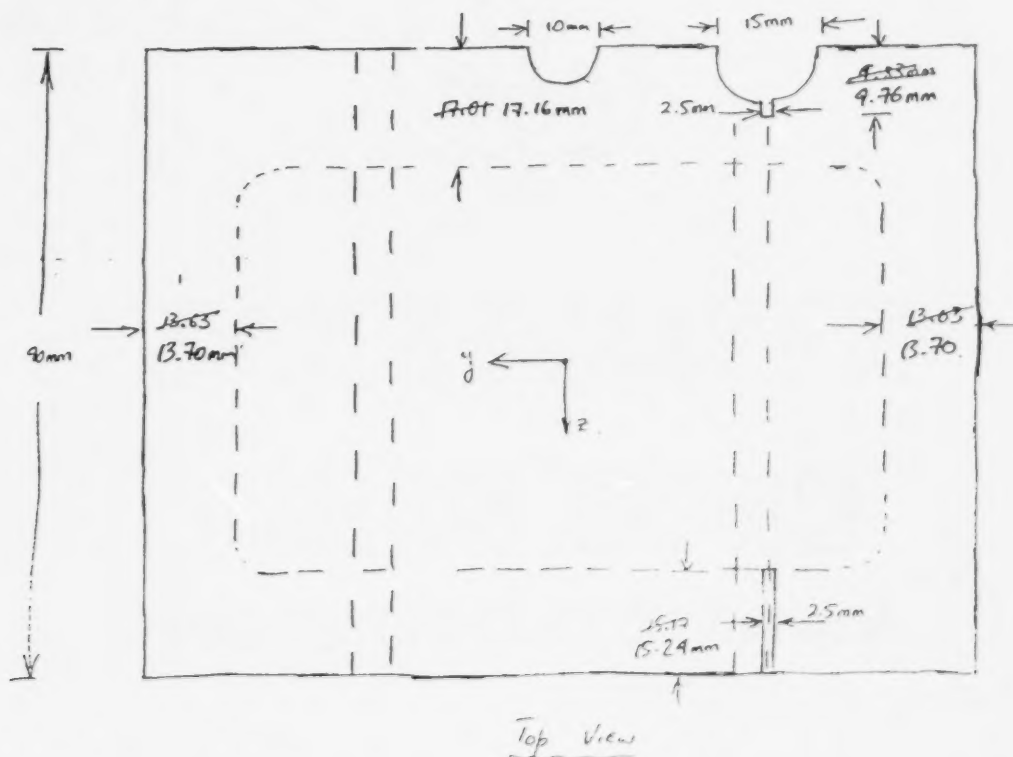
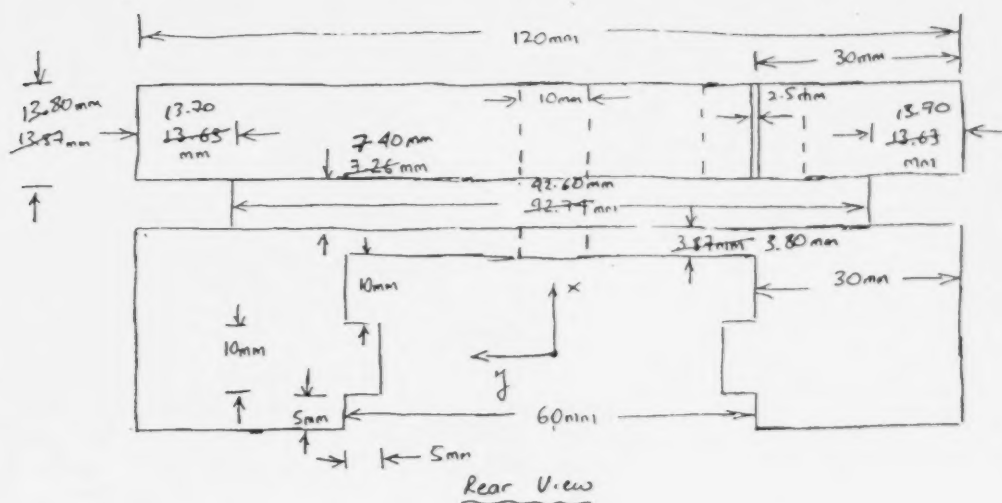
z-gradient



Rear and top views

1 of 3

x-gradient / quadrant 1



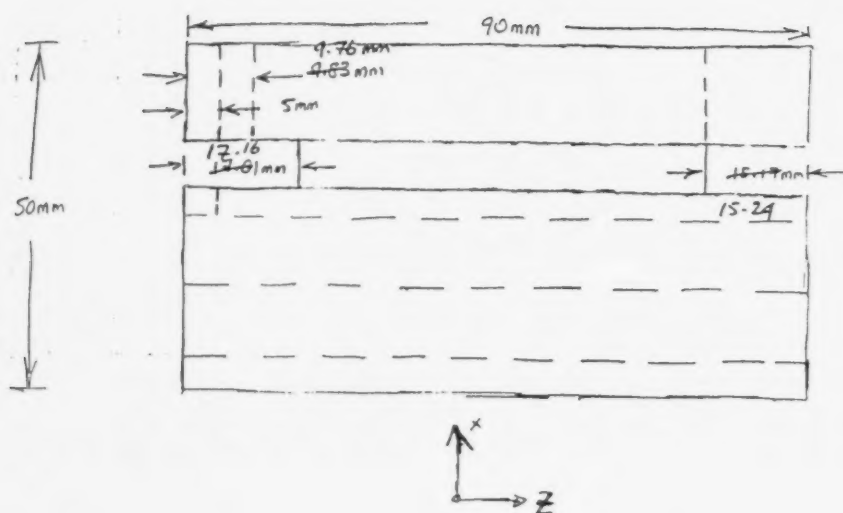
note All groove corners are rounded (to allow us to wind wire around it).

See additional diagram indicating desired curvature.

Side view

2 of 3

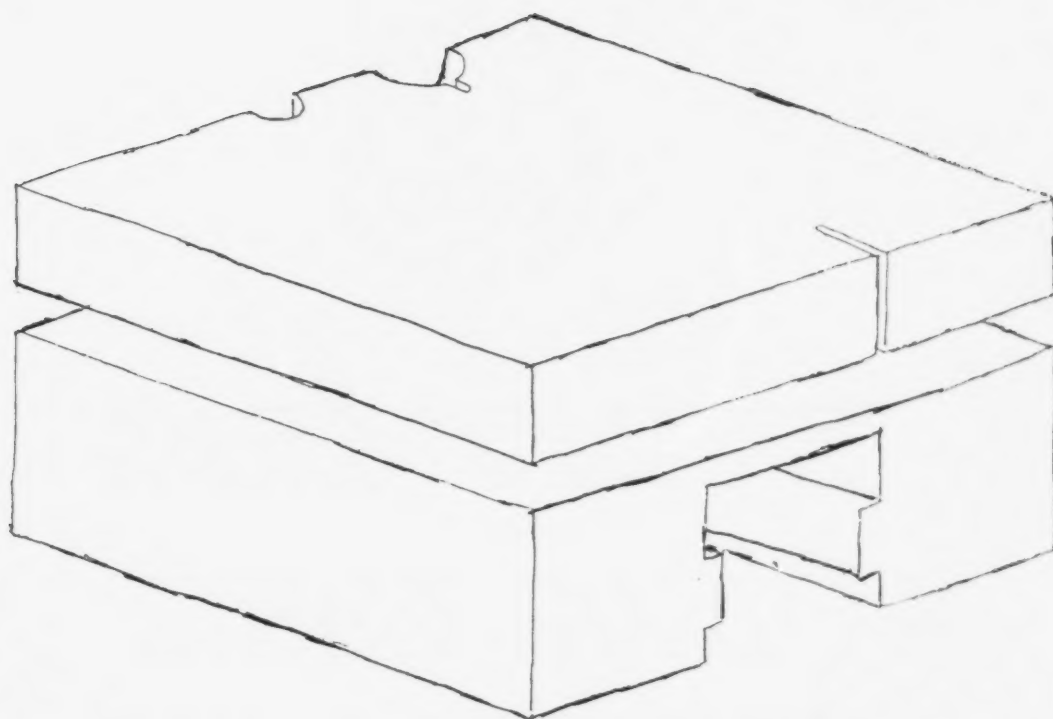
x-gradient / quadrant 1

Side View

3D view

3 of 3

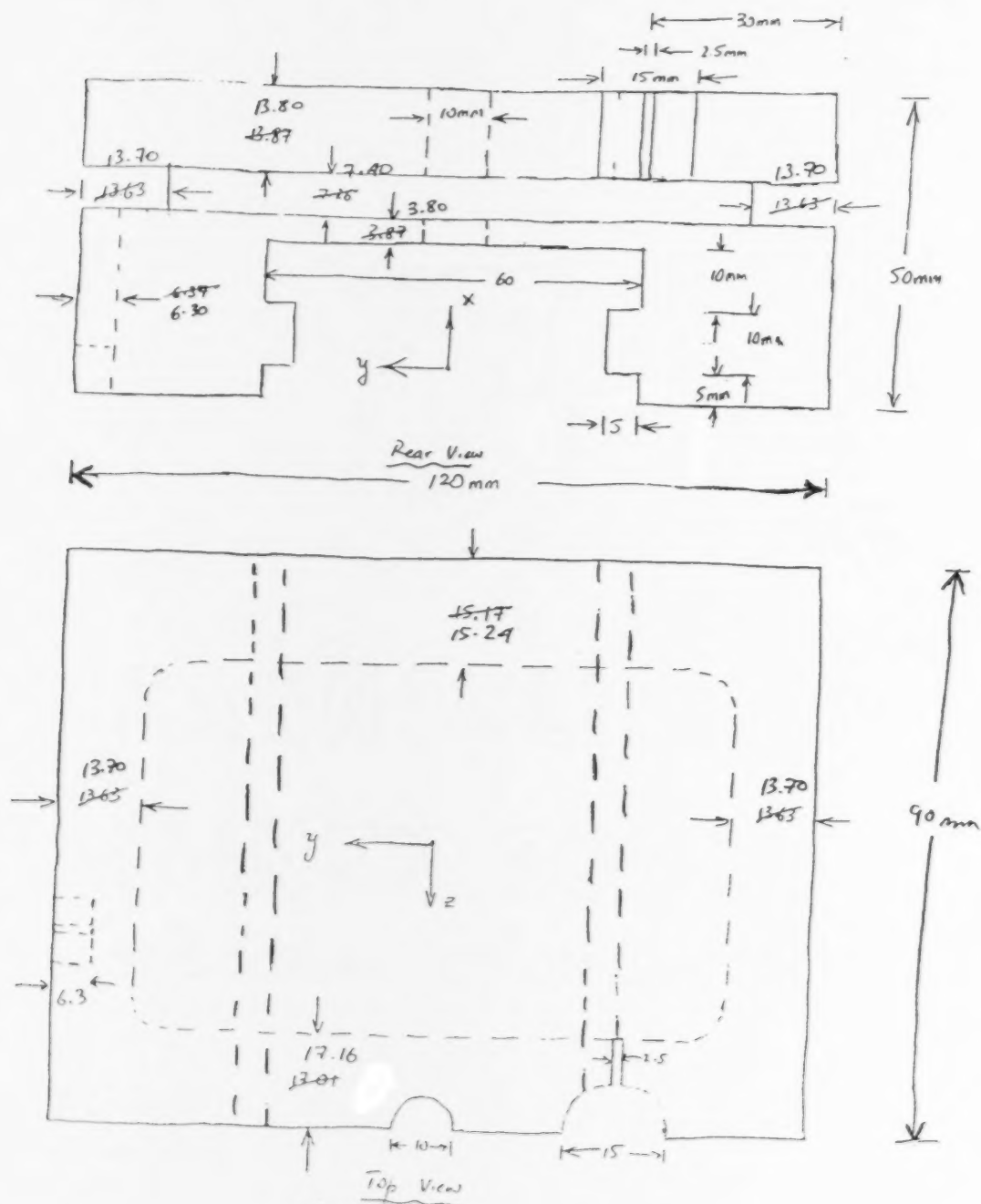
x-gradient / quadrant 1



Rear and top views

1 of 3.

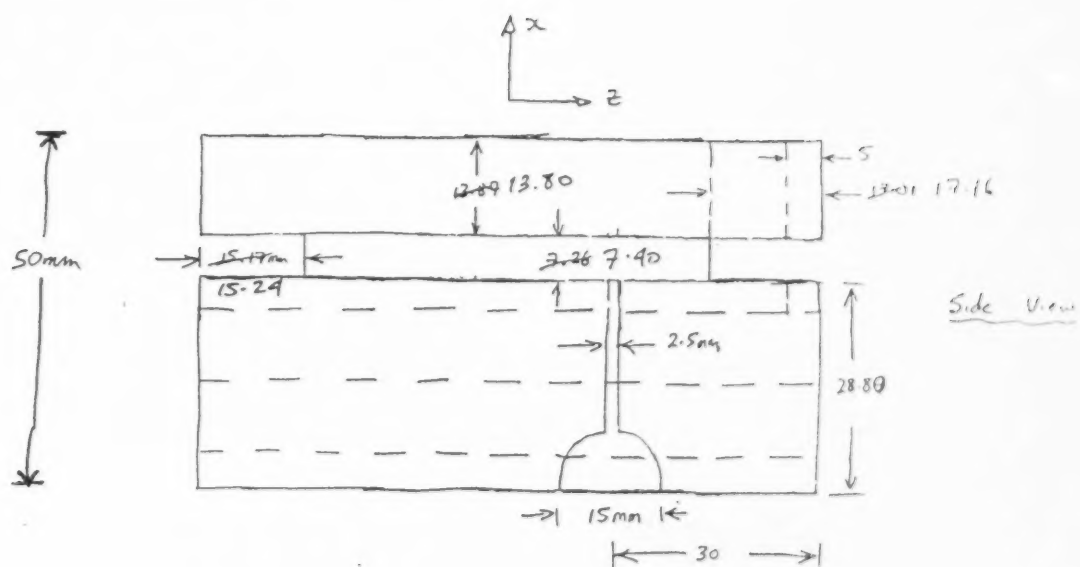
x-gradient / quadrant 2



Side view

2 of 3

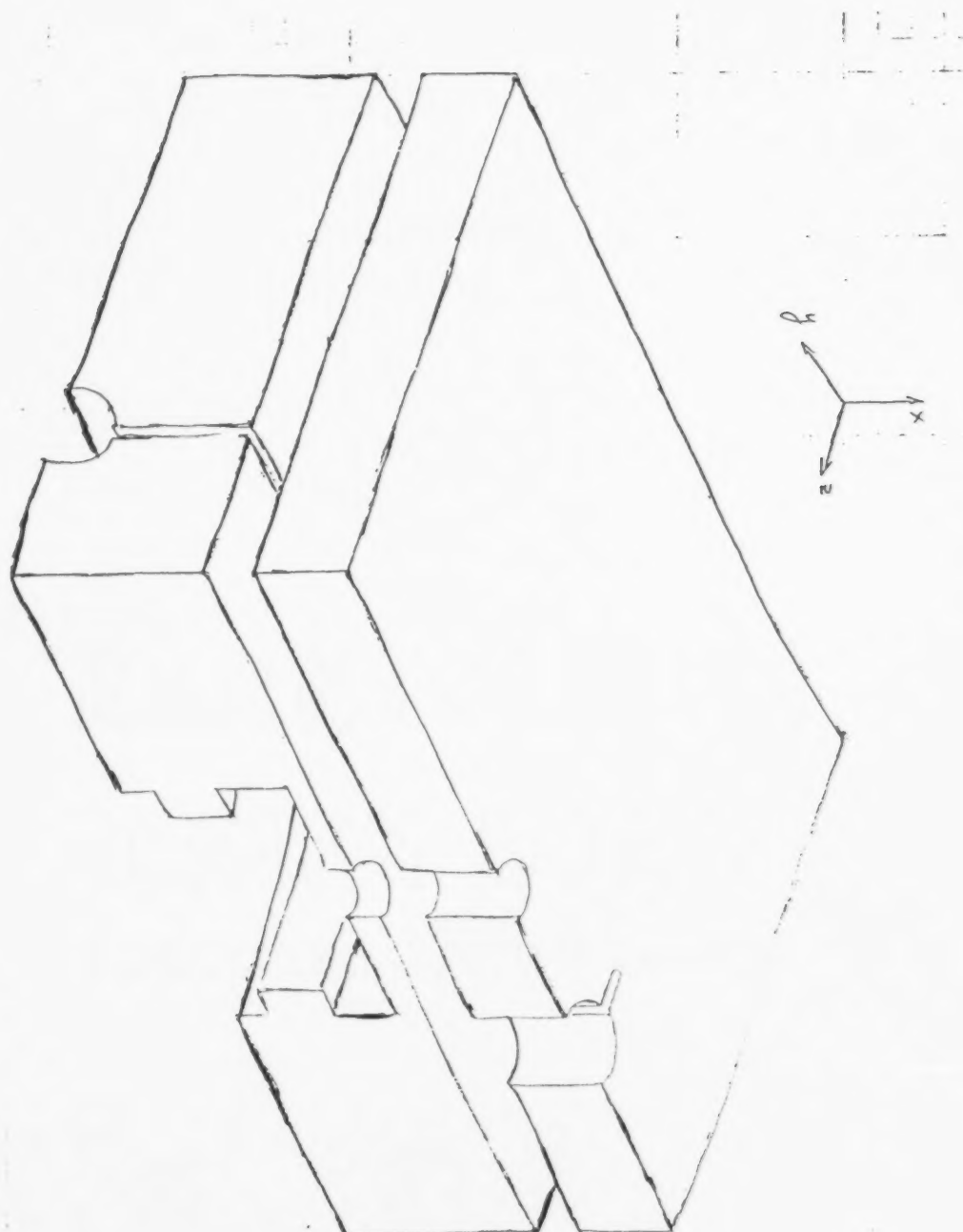
x-gradient / quadrant 2



3D view

3 of 3

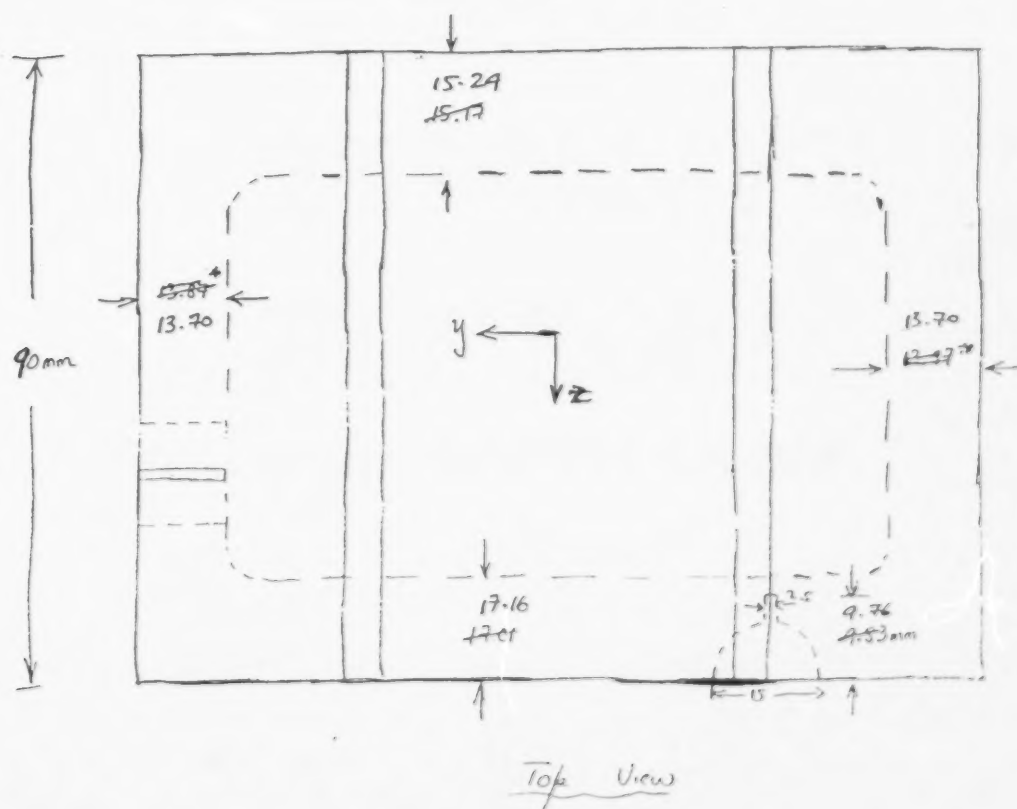
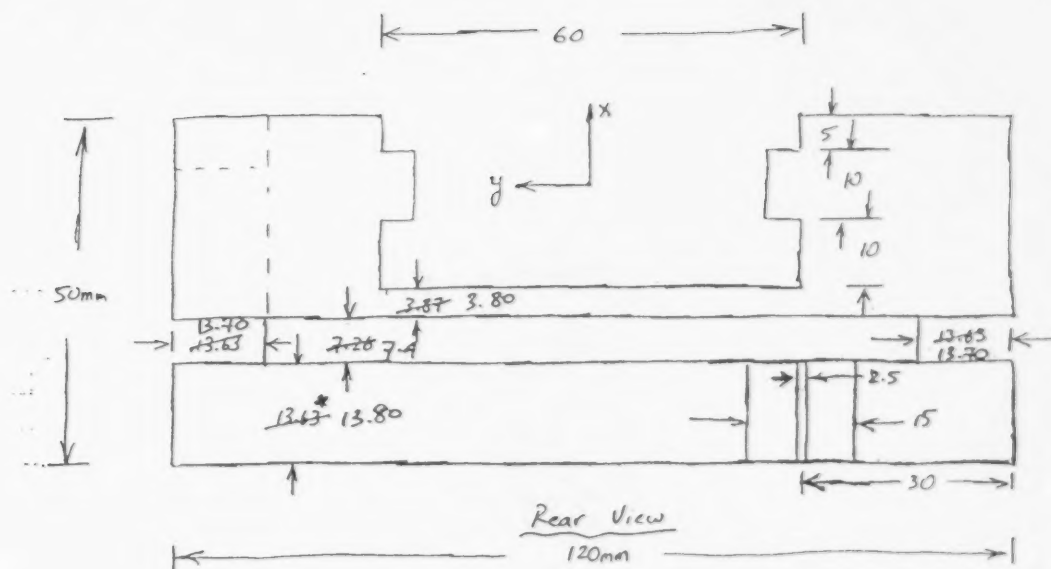
x-gradient / quadrant 2



Rear and top views

1 of 3

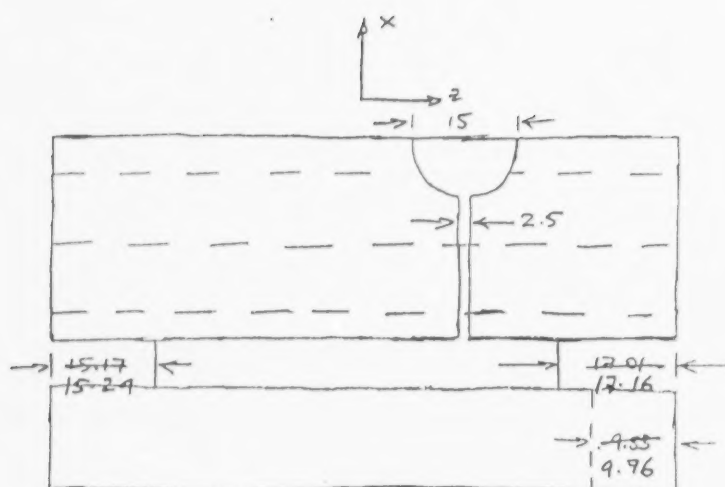
x-gradient / quadrant 3



Side view

2 of 3

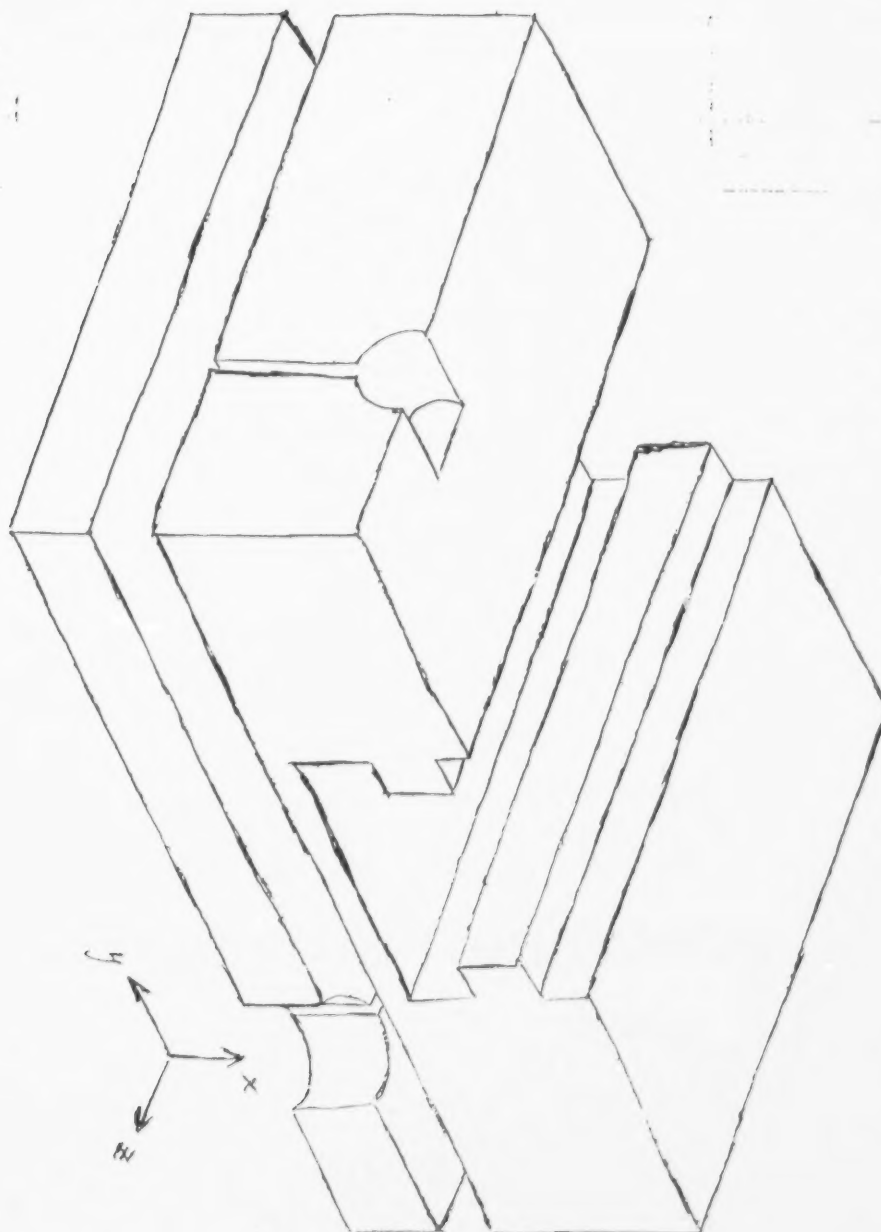
x-gradient / quadrant 3



3D view

3 of 3

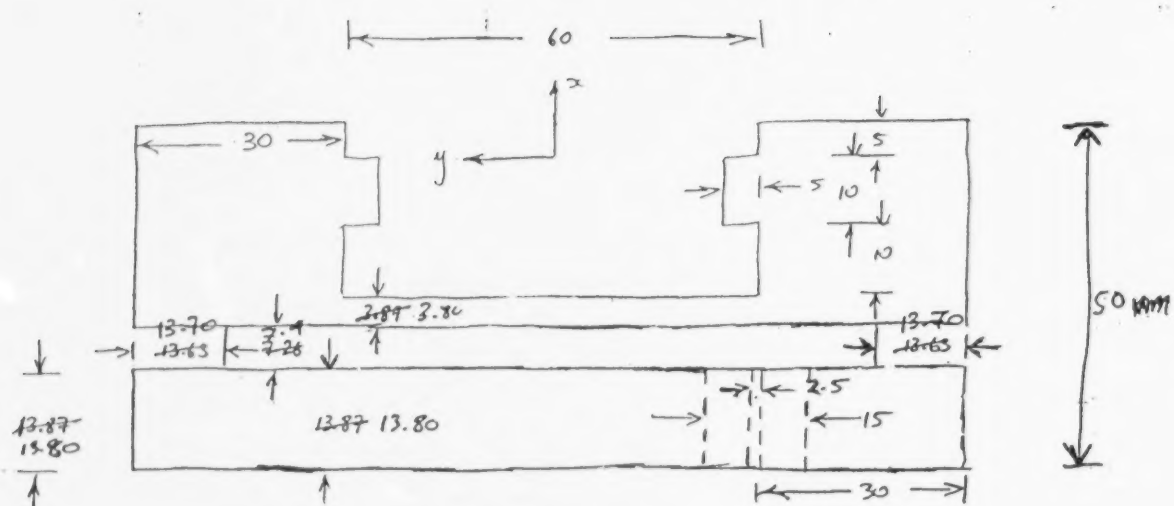
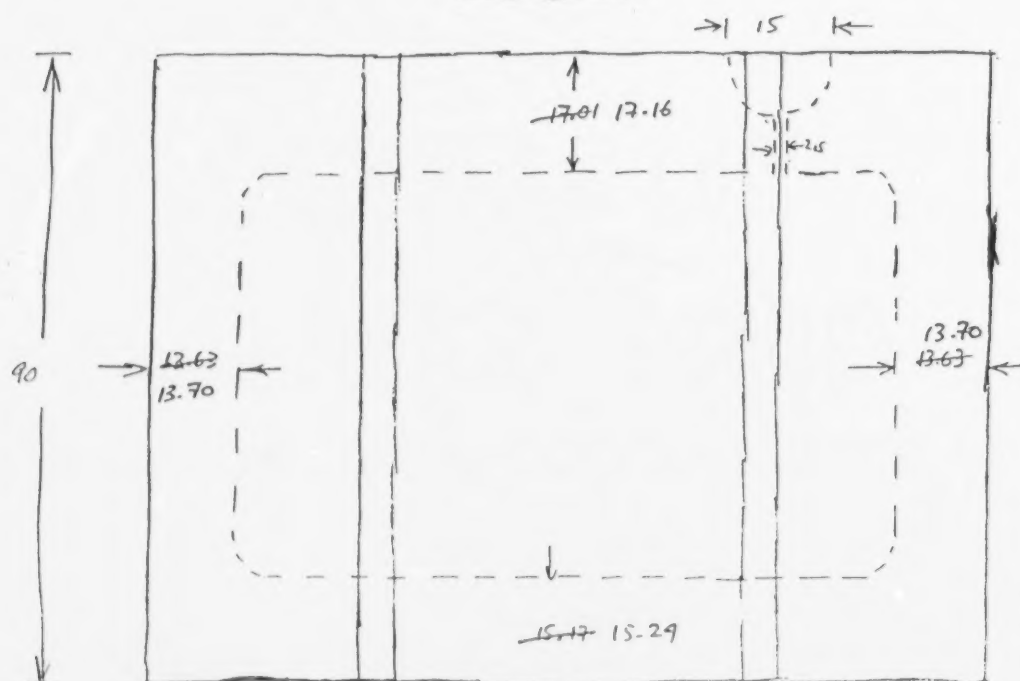
x-gradient / quadrant 3



Rear and top views

1 of 3

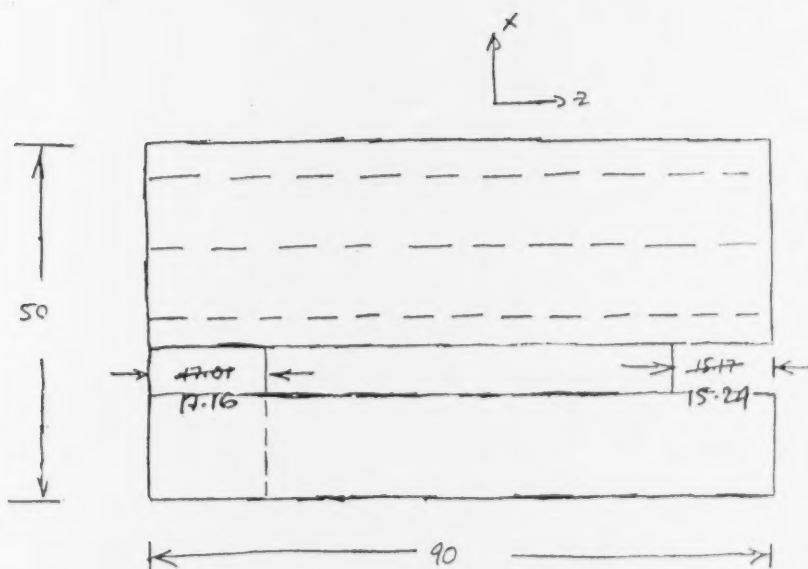
x-gradient / quadrant 4

Rear ViewTop View

Side view

2 of 3

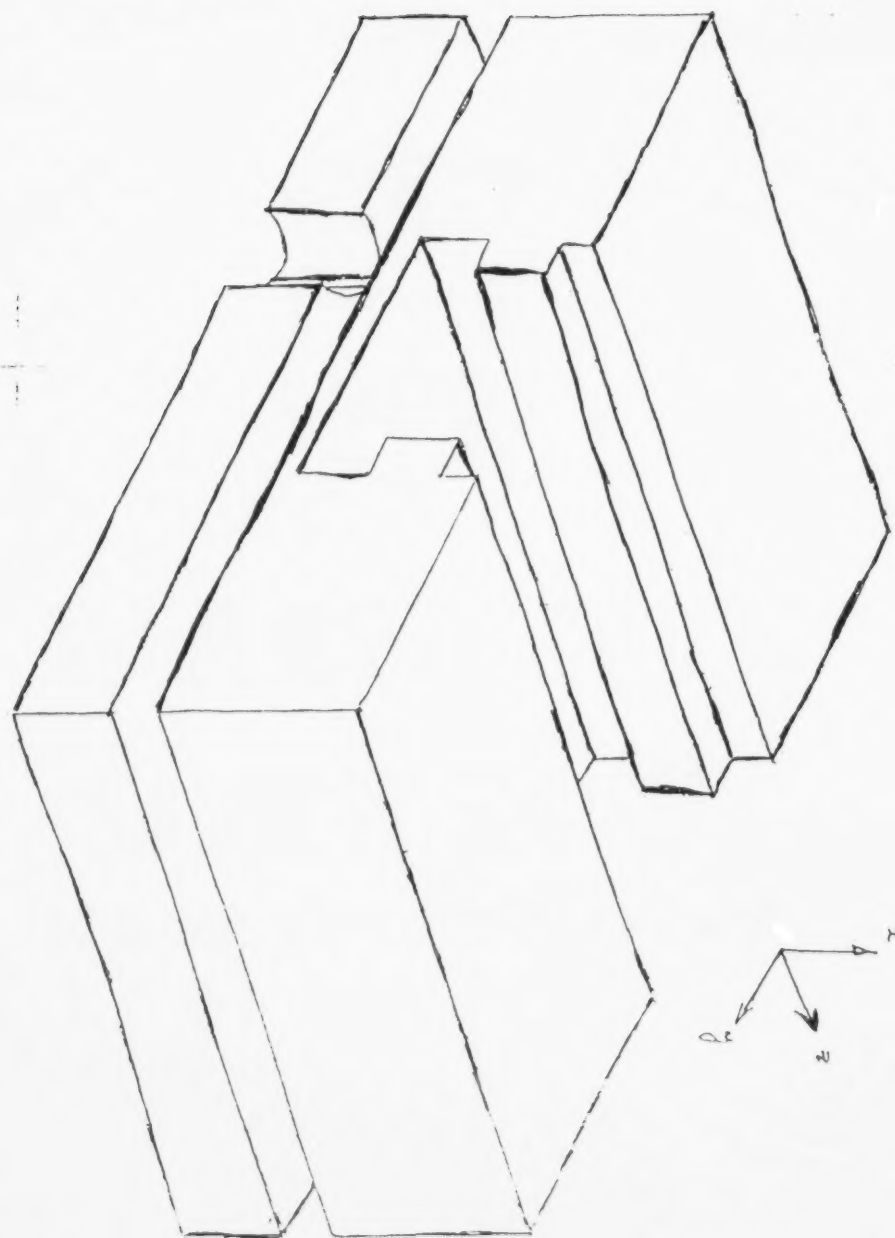
x-gradient / quadrant 4

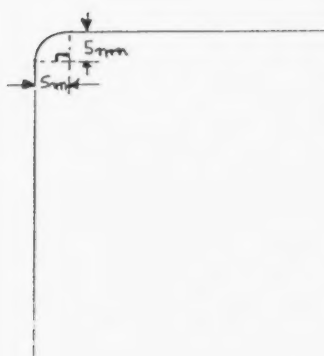


3D view

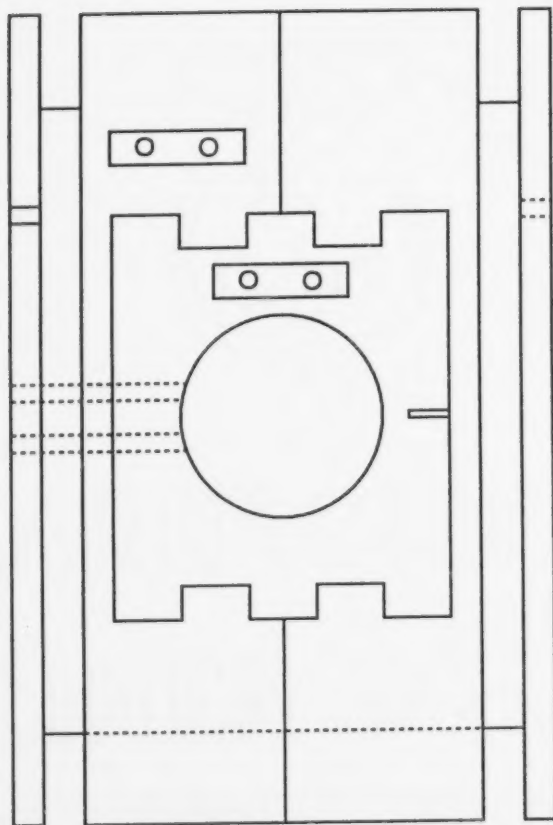
3 of 3

x-gradient / quadrant 4

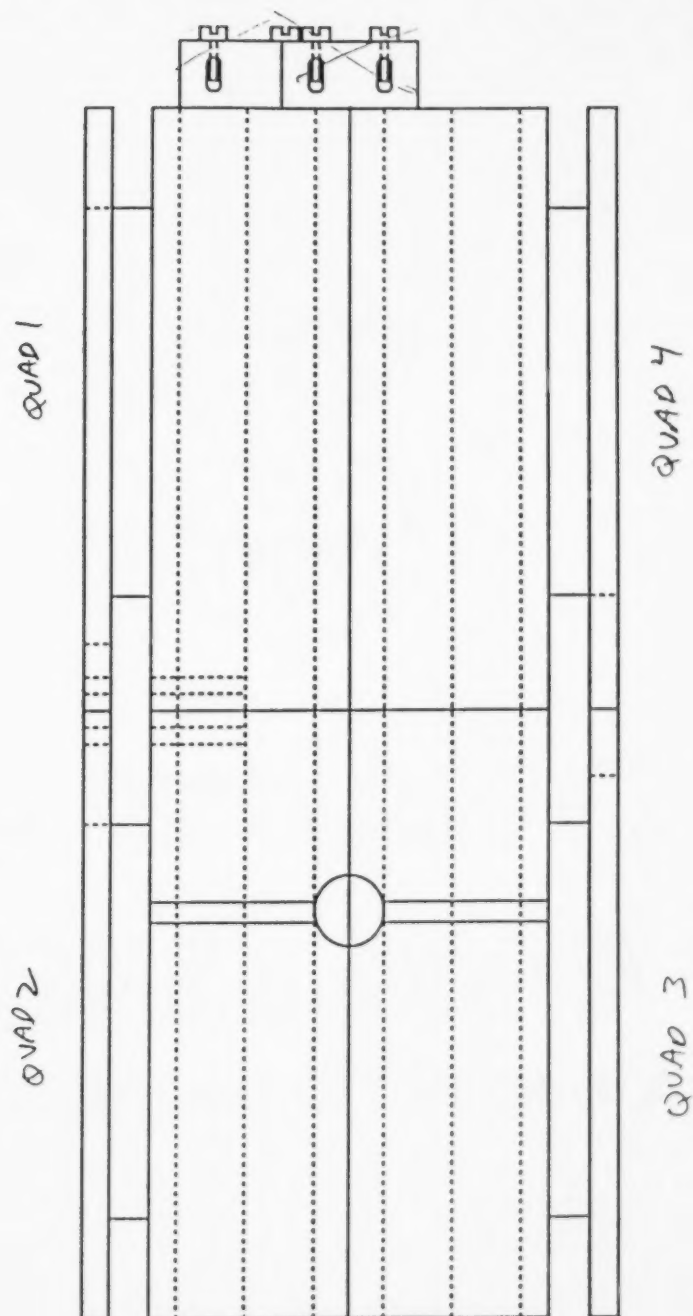




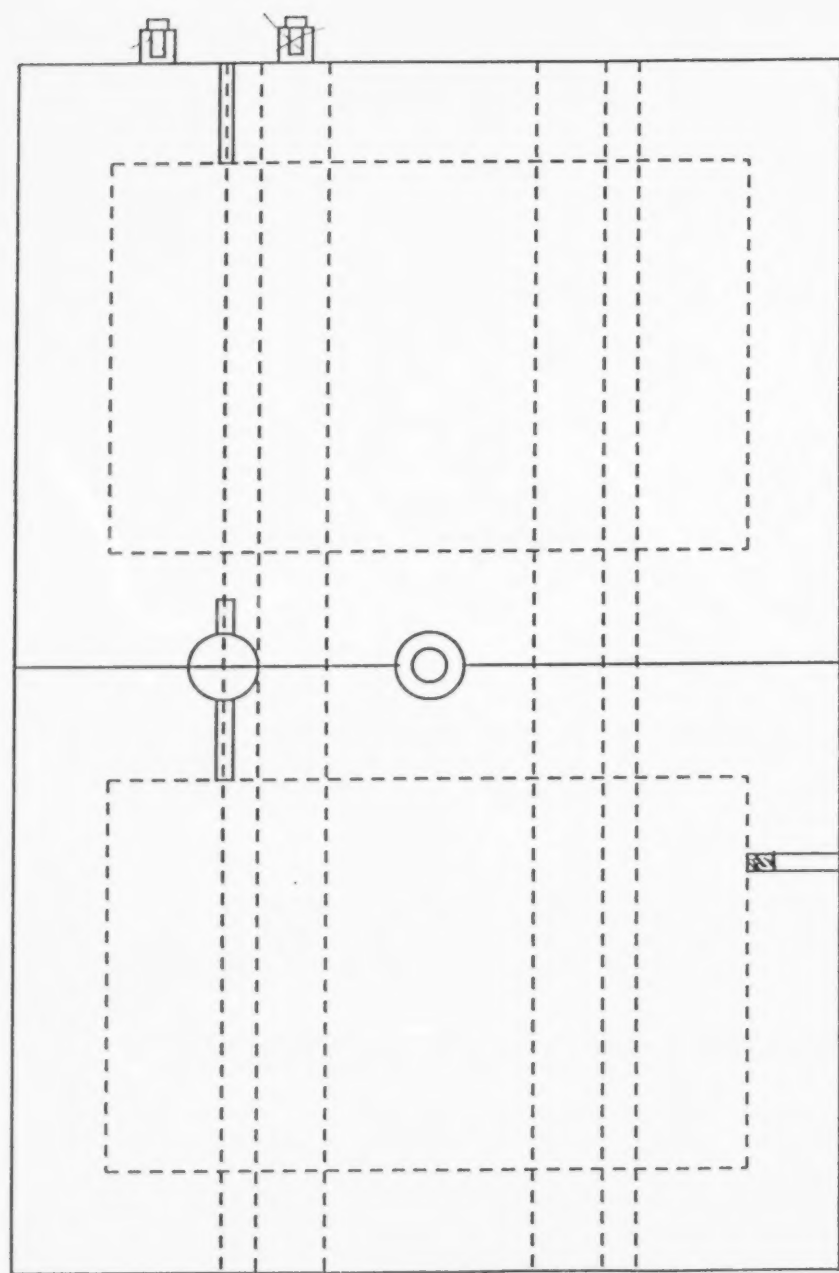
arcs for grooves
in quadrants 1 through 4



REAR VIEW



SIDE VIEW

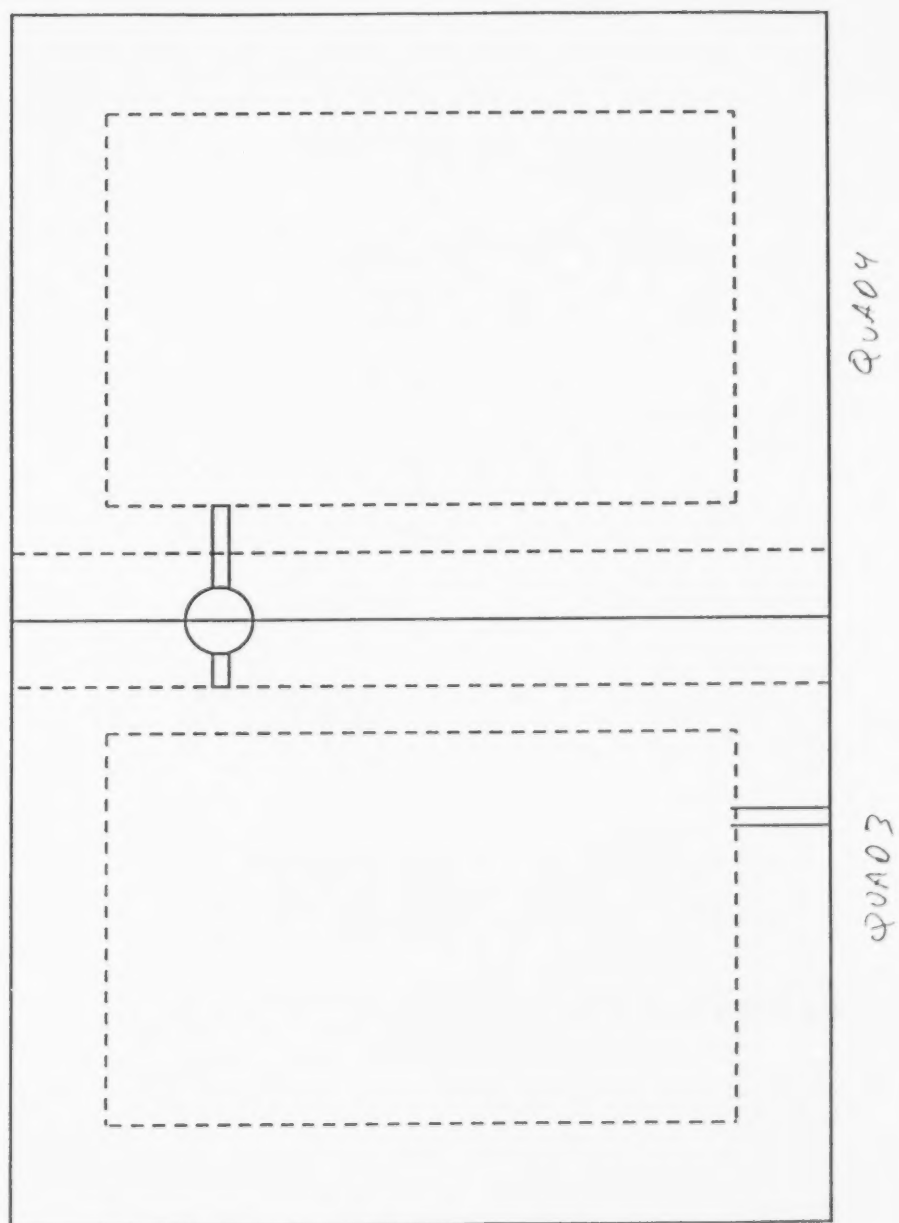


QUAD 1

QUAD 2

(top piece)

TOP VIEW



TOP VIEW (bottom pieces)

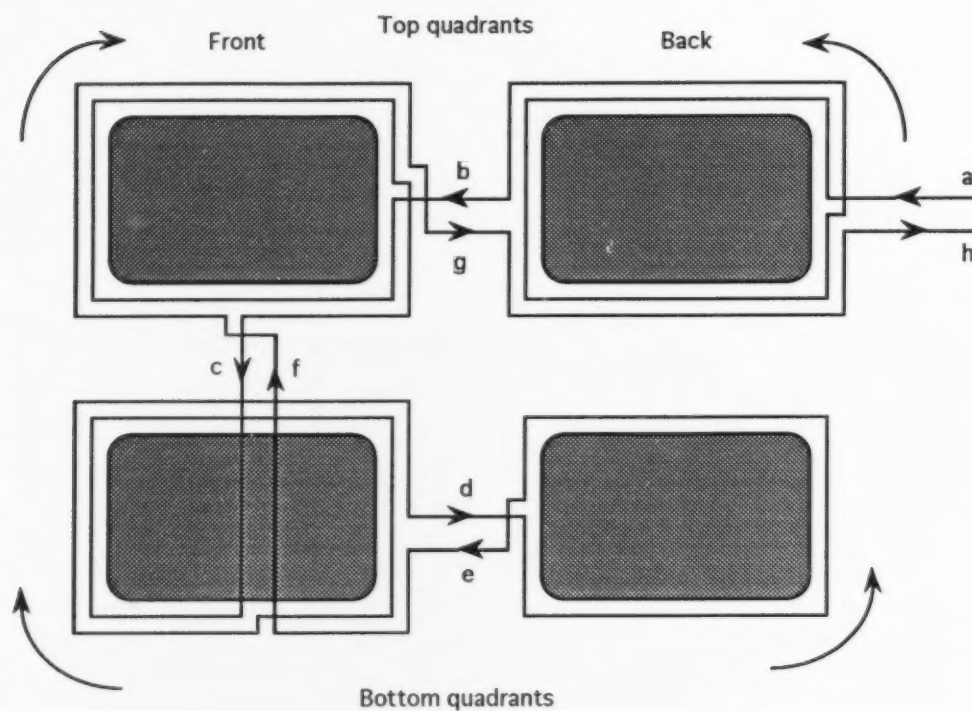


Figure A1.3 Pathways for the wires that were used for the x gradient. Seven pieces of wire (of varying length) were used and were soldered together at the junctions indicated by the letters b - g. The gradient wires were connected to the gradient drivers via a connector at a and h.